MEDICAL LIBRARY

MAY 22 1950

THE JOURNAL OF CLINICAL ENDOCRINOLOGY

Official Journal of the Association for the Study of Internal Secretions

VOLUME 10 NUMBER 4

APRIL 1950

DISPOSAL OF CHORIONIC GONADOTROPIN IN POSTPARTUM PERIOD . . 371
Carl E. Johnson, A. Albert and Robert B. Wilson

FERTILITY IN PARAPLEGIC MALES 381
Ernest Bors, Earl T. Engle, Robert C. Rosenquist and Victor H. Holliger

HYPOKALIEMIC, HYPOCHLOREMIC ALKALOSIS IN CUSHING'S SYNDROME 399
Robert Teabeaut, Frank L. Engel and Haywood Taylor

HYPERADRENALCORTICISM IN ACROMEGALY WITH INSULIN-RESISTANT DIABETES 410
William H. Daughaday, William E. Perry and Cyril M. MacBryde

IMPROVEMENT OF OVARIAN HYPEREMIA PREGNANCY TEST 423
Paul H. Fried and A. E. Rakoff

STABILITY OF EQUINE GONADOTROPIN 432
H. H. Cole, H. Goss and James Boda

NEW SIMPLE METHOD FOR MEASUREMENT OF URINARY I^{131} 437
A. Stone Freedberg, Alvin L. Ureles, Marvin VanDilla and Mary J. McManus

TREATMENT OF ESTROGEN DEFICIENCY WITH ESTRADIOL PELLETS . . 447
William H. Perloff

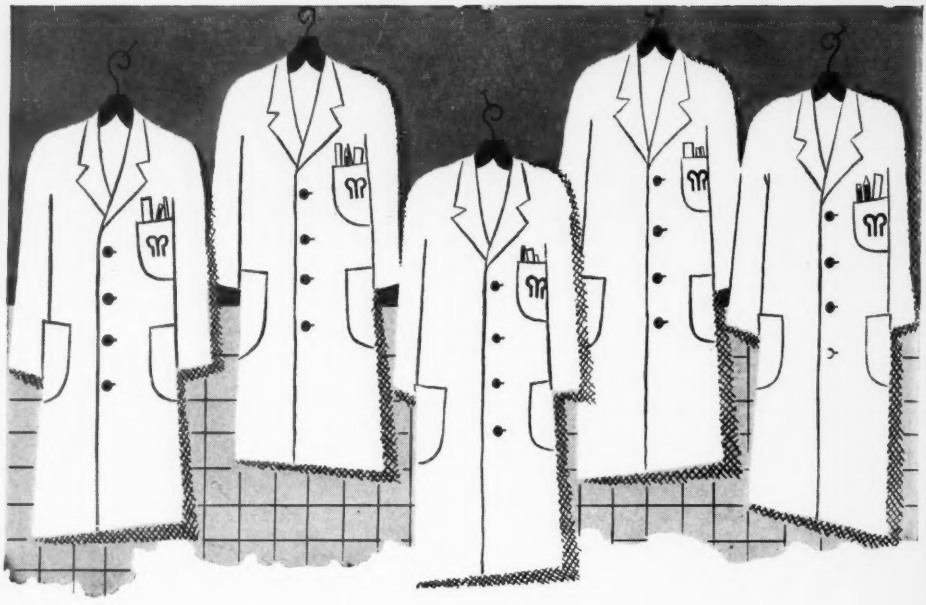
PREGNENOLONE. (AN ENDOCRINE REVIEW) 455
Edward Henderson, Marvin Weinberg and W. Allan Wright

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS
Program of the 1950 Annual Meeting 475

Postgraduate Course in Endocrinology, American College of Physicians . . . 476
Program of Laurentian Hormone Conference 477

Issued monthly for The Association for the Study of Internal Secretions by Charles C Thomas, Publisher. Annual subscription, $11.00; single copies, $1.00

ANNUAL MEETING • SAN FRANCISCO • JUNE 23 & 24, 1950



The mantles of the men who want a better thyroid

They seek better thyroid therapy and so they offer PROLOID

1. Extracted from fresh thyroid material and carefully assayed for potency so that the different thyroid batches can be balanced and then blended for more uniform ultimate potencies.

2. So purified of unwanted organic matter as to be free from the tell-tale odor of ordinary thyroid.

3. Standardized *metabolically* as well as chemically to guard the patient against metabolic fluctuations.

Available in ¼, ½, 1, 1½ and 5 grain tablets—bottles of 100, 500 and 5,000. Also as powder—1 oz. and 8 oz.

Proloid THE IMPROVED THYROID MEDICATION

CHILCOTT
Laboratories DIVISION OF The Maltine Company
MORRIS PLAINS, NEW JERSEY

The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 10 APRIL, 1950 NUMBER 4
Copyright 1950 by the Association for the Study of Internal Secretions

RENAL AND EXTRARENAL DISPOSAL OF CHORIONIC GONADOTROPIN IN THE IMMEDIATE POSTPARTUM PERIOD*

CARL E. JOHNSON, M.D., A. ALBERT, PH.D., M.D.
AND ROBERT B. WILSON, M.D.

From the Mayo Foundation, the Endocrinology Laboratory, Section of Clinical Physiology and the Section on Obstetrics and Gynecology, Mayo Clinic, Rochester, Minnesota

THE disposal of chorionic gonadotropin in the human being has been a matter of conjecture for some time. The method commonly employed in studies on this question has consisted of injecting a known amount of the principle into men or nonpregnant women and of determining what proportion of the administered dose appeared in the urine. Friedman and Weinstein (1) found that in men not more than 20 per cent of the injected dose was excreted by the kidneys. However, in nonpregnant women, Bradbury and Brown (2) reported an instance in which more than 50 per cent of the administered dose appeared in the urine. Allowing for slower rates of elimination both before and after the period during which urinary assays were made, they expressed the belief that most of the dose was excreted and that relatively little destruction or utilization of chorionic gonadotropin occurred in the body. These views were shared by Lloyd and associates (3) who also reported a case in which 30 per cent of the injected dose was eliminated by the kidneys. However, they listed

Received for publication September 20, 1949.

* Abridgment of thesis submitted by Dr. Johnson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Obstetrics and Gynecology.

other cases in which no chorionic gonadotropin was found in the urine after parenteral administration of 5,000 I. U. There is also much work on animals on this problem, summarized by Zondek and Sulman (4), but inasmuch as chorionic gonadotropin is a foreign substance to animals other than primates, the results obtained with rats, dogs, rabbits and other nonprimate mammals are not necessarily applicable to human beings.

The exact fate of chorionic gonadotropin is of some practical as well as theoretic importance since knowledge of its excretion is essential in attempts at replacement therapy during pregnancy or for an understanding of certain abnormal phases of pregnancy such as the toxemias, in which an abnormality in the metabolism of this principle is said to exist (5). The foregoing results are variable and leave doubt as to the exact role of the kidneys in the metabolism of this principle. Furthermore, they were obtained in nonpregnant patients, and it is problematic that such results would necessarily be applicable to the metabolism of the hormone during the pregnant state.

Because of these considerations, we have investigated this problem by a different approach. Immediately on delivery of the placenta, samples of blood and urine were obtained serially at varying intervals for several days, and determinations of the principle were made. From the initial blood concentration, the total hormone in serum was easily computed. Since the placenta, assumed to be the sole source of the principle, was no longer present, no further chorionic gonadotropin could be delivered to the blood. Furthermore, no "placental utilization" of the principle could occur. Thus, the urine remained as a chief route of disposal of the hormone and the fraction of the total serum hormone appearing in the urine could then be determined. By this method and by supportive data from analysis of the blood and urine curves, the renal and extrarenal disposal of chorionic gonadotropin in the immediate postpartum period could be established.

MATERIAL AND METHODS

Fifteen pregnant women volunteered for this study. Most of these patients were healthy women, and in none was there any endocrine abnormality associated with pregnancy. A brief description of these patients is included in the appendix. At the time of placental delivery, either by cesarean section or by the usual route, a sample of blood was obtained by venipuncture in the antecubital space. A catheter was inserted into the bladder, and urine was collected for several days. At varying intervals, blood samples were obtained, and urine samples corresponding to the same intervals of time were taken for assay. Determination of chorionic gonadotropin was performed according to the method of Albert (6). Total serum was estimated at 3 liters per patient. Actual serum volume during preg-

nancy by direct determination is apparently variable, increasing progressively toward onset of labor (7)

RESULTS

In the first 6 patients, blood and urine samples were obtained every twenty-four hours. These results, shown in Table 1, served to delimit the time of observation, and also indicate the order of magnitude of the urinary excretion of chorionic gonadotropin following removal of the placenta. It appeared that the serum would most probably be cleared of the principle within twenty-four hours, and that the urine would excrete minimal amounts within twenty-four to forty-eight hours.

TABLE 1. CHORIONIC GONADOTROPIN (I. U.) IN TOTAL SERUM (I. U./CC. SERUM, × 3,000 CC.) AND IN URINE FOLLOWING DELIVERY OF THE PLACENTA

Patient	Serum				Urine		
	Time in hours after delivery				Time in hours after delivery		
	0	24	48	72	0–24	24–48	48–72
1	27,000	Neg.	Neg.	Neg.	3,708	777	Neg.
2	11,000	Neg.	Neg.	Neg.	Neg.	Neg.	
3	50,700	Neg.	Neg.		7,050	Neg.	
4	34,110	11,100	Neg.		1,890	Neg.	
5	29,100	Neg.			2,070	Neg.	
6	18,600	Neg.	Neg.	Neg.	1,015	Neg.	Neg.

Accordingly, in the next 9 patients, samples of blood and urine were obtained at various intervals during two days. The results (Table 2) showed that after twelve hours no positive reactions were obtained for the principle in serum, and that in most instances the serum gonadotropin had rapidly disappeared within twelve hours so that the extinction point of the bioassay (3 I U /cc. serum) had been reached. The urine contained measurable amounts of the principle for a longer period than serum, but the extinction point of the assay (0.3 I. U./cc. urine) was reached within thirty-six hours after the removal of the placenta. A graphic summary of these results is given in Figure 1, which shows for each of the 15 patients the total amount of the principle in the serum immediately after removal of the placenta and the total amount appearing in the urine subsequently. The mean values were $36,245 \pm 6,000$ I. U. and $2,105 \pm 480$ I. U., respectively. Thus, 5.8 per cent of the principle in the serum was excreted by the kidneys.

Five of the studies (on patients 7, 8, 13, 14 and 15) made possible further analysis (8, 9). The curves of disappearance of the principle from the serum

TABLE 2. CHORIONIC GONADOTROPIN (I. U.) IN TOTAL SERUM (I. U./CC. SERUM, × 3,000 CC.) AND IN URINE AT VARIOUS INTERVALS FOLLOWING DELIVERY OF THE PLACENTA*

Patient		*t*, hours following delivery of placenta										
		0	3	4	6	8	9	12	18	24	36	48
7	S†	63,000			29,100			Neg.		Neg.		
	U†				1,435			Neg.		Neg.		
8	S	50,700	34,200		18,000		15,300	15,300		Neg.		
	U		540		735		303	162		Neg.		
9	S	31,200		Neg.		Neg.						
	U			608		Neg.		Neg.				
10	S	18,660			Neg.			Neg.				
	U				230			Neg.				
11	S	15,300			Neg.			Neg.				
	U				383			446	Neg.			
12	S	11,100		Neg.								
	U			128		555			Neg.			
13	S	24,900	11,100		Neg.			388		Neg.		
	U		1,015	316			555					
14	S	71,000		21,000		18,000		Neg.				
	U			2,255		1,068				1,377	Neg.	Neg.
15	S	87,300		19,500				Neg.				
	U			648				1,436		Neg.		Neg.

* The entries for serum represent the estimated total amount of gonadotropin in the serum at time *t*. The entries for urine represent the estimated amount of gonadotropin excreted into the urine between the time of previous estimate and the time shown.

† S = Serum. U = Urine.

of these patients are shown in Figure 2. Assuming a logarithmic decrease of concentration, the proportional rate of disappearance (r) of the principle from the blood can be estimated by

$$\frac{\log b_1 - \log b_2}{0.434 \times t},$$

where b_1 and b_2 are the ordinates of two points (I. U. per cubic centimeter of serum) on the curve, and t is the time interval in hours between these two points. Similar calculations can be applied to the appearance of the principle in the urine, and two parameters (the renal excretion rate and the

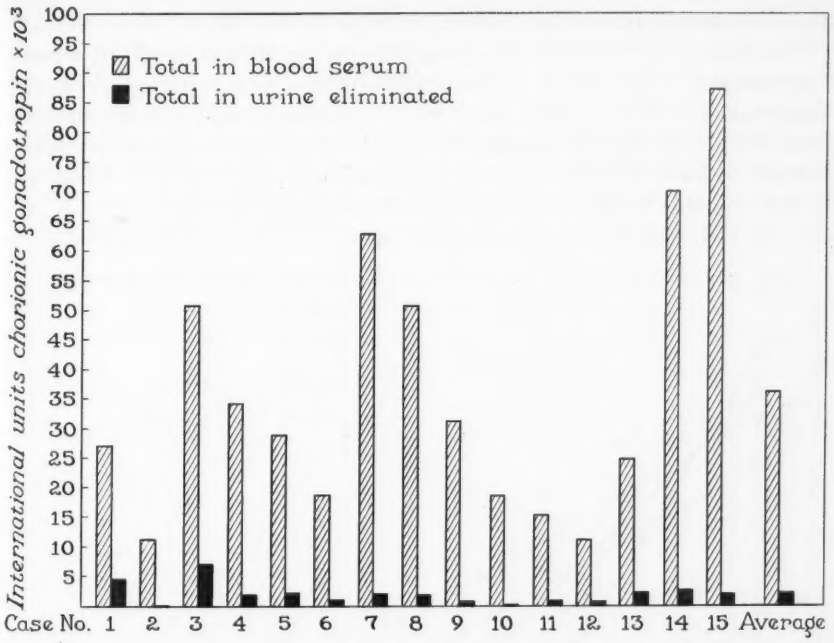


FIG. 1. Summary chart of results for each of the 15 patients. Hatched columns represent total chorionic gonadotropin in the serum at delivery; solid columns represent the total amount of the principle appearing in the urine after delivery.

quantity of chorionic gonadotropin eventually appearing in the urine) can be determined. These two parameters in the present case are highly tentative, for reasons beyond the scope of the present paper, and are used here only to indicate a rough order of magnitude. A further calculation of significance is the determination of renal clearance, which can be obtained in the conventional manner by dividing the estimated amount of gonadotropin excreted into the urine, during a period, by the average blood concentration during that period.

$$\text{Clearance} = \frac{Qu.2 - Qu.1}{\overline{C}b},$$

where $Qu.1$ is the amount excreted into the urine up to t_1, $Qu.2$ is the amount excreted up to t_2 and $\overline{C}b$ is the average concentration in the blood during the interval $t_1 - t_2$. If the concentration in the blood is decreasing logarithmically in this period, the average concentration is given by

$$\overline{C}b = \frac{(Cb_1 - Cb_2) \times 0.4343}{\log Cb_1 - \log Cb_2}.$$

The various calculations are shown in Table 3.

The data in Table 3 are of especial interest in that they afford a more comprehensive view of the disposal of chorionic gonadotropin. From the disappearance rate r, which gives the proportional rate of disappearance from the blood, may be calculated the time required for half of the initial amount to have disappeared and this is given by $t50 = 0.693/r$. These are shown in Table 3. The mean for all the cases was 3.5 hours.

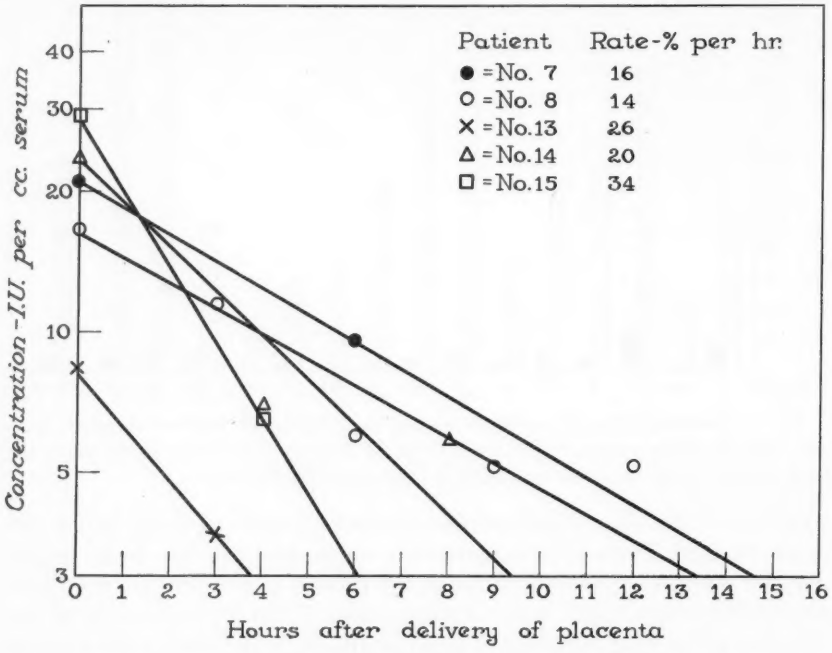


FIG. 2. Curves of the disappearance of chorionic gonadotropin in the serum following delivery of the placenta.

The mean time at which the principle could no longer be detected in the serum by our assay method was 9.3 hours. This value was determined directly by the intercept of blood serum curves on the time axis at a concentration of 3 I. U. per cubic centimeter of serum. This concentration is the extinction point of the assay and the corresponding time, 9.3 hours, is therefore the extinction time. If the disappearance of the principle from blood were due entirely to renal excretion, this extinction time should correspond to a similar value obtained by use of the data on renal clearance. With a mean clearance of 0.47 cc. per minute, which is not essentially different from that determined by Gastineau, Albert and Randall (10) dur-

ing the entire pregnancy, an average serum volume of 3 liters would be cleared of its chorionic gonadotropin in 106 hours. Applying the ratio $b_0/3$ I. U. where b_0 is the concentration in I. U. per cubic centimeter of serum for each of the patients at zero time, a mean ratio of 6:1 was obtained. Thus, five sixths of the total serum, or approximately 2,500 cc., would be cleared of the principle to correspond to the extinction value of the assay. This volume of serum would require an extinction time of eighty-nine hours ($2{,}500/0.47\times60$) for removal of the principle, if the only

TABLE 3. DISAPPEARANCE OF CHORIONIC GONADOTROPIN FROM THE SERUM AND ITS RENAL EXCRETION

Patient	r* per cent per hour	Half time, hours	Quf,† per cent	Renal excretion rate (Quf, $\times r$), per cent per hour	Renal clearance, cc. per minute
7	16	4.33	3.75	0.60	0.28
8	14	4.95	4.00	0.56	0.29
13	26	2.67	9.00	2.34	0.99
14	20	3.46	6.10	1.22	0.60
15	34	2.04	4.50	1.53	0.18
Mean	22	3.50	5.47	1.25	0.47

* Rate of disappearance of the principle from serum.

† Asymptotic quantity of the principle eventually appearing in the urine, as per cent of total principle in the serum. The volume of serum in this instance was estimated conservatively at 5 per cent of the body weight at term.

route of disposal from blood were elimination in the urine. However, by direct observation the extinction time of the principle in the blood was found to be 9.3 hours, a value which is grossly incompatible with the time (89 hours) that would be required if all the principle were eliminated via the kidneys.

COMMENT

The conclusion that not more than 5.8 per cent of the serum chorionic gonadotropin is eliminated by the kidneys is in obvious variance with the previous estimations (20 per cent to about 100 per cent) by the method of injecting known amounts of the principle and measuring the proportion that appears in the urine. Our value of 5.8 per cent, however, is a very conservative estimate, since if the volume of distribution of the principle, which is not known, proves to be larger than the serum volume, the percentage of the hormone excreted in the urine would be proportionally smaller. Although the kinetic analysis was feasible in only 5 of the cases, and represents only rough approximations, it would not appear possible to

explain such a large difference in order of magnitude as given in Table 3 for renal and extrarenal disposal rates on the basis of small numbers of cases.

It does not seem possible to reconcile these data with the results of injection experiments except on the ground that the behavior of the principle may be different in pregnancy than in the nonpregnant state. In the immediate postpartum period, our results show that only a small proportion of the active principle is eliminated by the kidneys, and the remainder is disposed of by some other route. The nature of the extrarenal disposal of active principle is unknown. It probably does not consist of elimination of active principle by some other excretory channel such as feces, but presumably represents endogenous inactivation or destruction of the molecule.

SUMMARY

The renal and extrarenal disposal of chorionic gonadotropin was determined in 15 pregnant women during the immediate postpartum period, by simultaneous blood and urine assay following delivery of the placenta. Only 5.8 per cent of the principle in serum was eliminated in active form in the urine, the remainder presumably being inactivated or destroyed in the body.

APPENDIX OF PERTINENT DATA ON SUBJECTS

Case 1.—The patient was a white woman, aged 23 years, para 2. Her weight at delivery was 174 pounds (about 79 Kg.). The placenta was delivered intact. The postpartum course was normal.

Case 2.—The patient was a white woman, aged 25 years, para 3. Her weight was 145 pounds (about 66 Kg.). The placenta was delivered intact. The postpartum course was normal.

Case 3.—The patient was a white woman, aged 22 years, para 1. Her weight was 156 pounds (about 71 Kg.). The placenta was delivered intact by section because of inertia after thirty-eight hours of labor. The postoperative course was uneventful.

Case 4.—The patient was a white woman, aged 24 years, para 2. Her weight was 107 pounds (about 48 Kg.). She had had renal disease since the age of 4 years. A previous section had been performed at age 20 years because of chronic pyelonephritis and cephalopelvic disproportion. In the present pregnancy, blood urea rose to 140 mg. per 100 cc. Albuminuria, pyuria and erythrocyturia were present. After section, blood urea became normal. The placenta was delivered intact by section. The postoperative course was uneventful.

Case 5.—The patient was a white woman, aged 22 years, para 3. Her weight was 157 pounds (about 71 Kg.). The placenta was delivered intact by elective section because of two previous sections.

Case 6.—The patient was a white woman, aged 23 years, para 2. Her weight was about 155 pounds (70 Kg.). The placenta was delivered intact by section because of placenta previa.

Case 7.—The patient was a white woman, aged 21 years, para 2. Her weight was 131 pounds (about 59 Kg.). Elective section was performed because of previous section elsewhere.

Case 8.—The patient was a white woman, aged 22 years, para 1. Her weight was 138 pounds (about 63 Kg.). The placenta was delivered intact. The postpartum course was normal.

Case 9.—The patient was a white woman, aged 35 years, para 4. Her weight was 160 pounds (about 73 Kg.). Her postpartum course after delivery of the intact placenta was normal.

Case 10.—The patient was a white woman, aged 32 years, para 1. Her weight was 168 pounds (about 76 Kg.). The placenta was delivered intact. The postpartum course was normal.

Case 11.—The patient was a white woman, aged 29 years, para 1. Her weight was 165 pounds (about 75 Kg.). The placenta was delivered intact. The postpartum course was normal.

Case 12.—The patient was a white woman, aged 37 years, para 6. Her weight was 166 pounds (about 75 Kg.). Forty-eight hours before delivery, the patient noticed absence of fetal movement. She was seen at the Mayo Clinic ten hours before delivery, when no fetal heart sounds could be heard. Labor was spontaneously initiated, and a dead infant was delivered. The placenta was intact. The postpartum course was normal.

Case 13.—The patient was a white woman, aged 29 years, para 3. Her weight was 186 pounds (about 84 Kg.). Elective section was done because of three previous sections for cephalopelvic disproportion. The placenta was intact. The postoperative course was normal.

Case 14.—The patient was a white woman, aged 39 years, para 1. Her weight was 146 pounds (about 66 Kg.). Section was performed because of cephalopelvic disproportion. The placenta was intact. The postoperative course was normal.

Case 15.—The patient was a white woman, aged 18 years, para 3. Her weight was 148 pounds (about 67 Kg.). Elective section was performed because of previous section. The placenta was intact. The postoperative course was normal.

REFERENCES

1. Friedman, M. H., and Weinstein, G. L.: The excretion of gonadotropin by normal human males after the ingestion and injection of extracts of pregnancy urine, *Endocrinology* **21**: 489–494 (July) 1937.
2. Bradbury, J. T., and Brown, W. E.: Absorption and excretion of chorionic gonadotropin administered intramuscularly in women, *J. Clin. Endocrinol.* **8**: 1037–1042 (Dec.) 1948.
3. Lloyd, C. W.; Hughes, E. C.; Eva, M. L., and Lobotsky, J.: The urinary excretion of chorionic gonadotropin by human females following parenteral administration of aqueous or beeswax solutions, *J. Clin. Endocrinol.* **9**: 268–274 (Mar.) 1949.
4. Zondek, Bernhard, and Sulman, Felix: The mechanism of action and metabolism of gonadotropic hormones in the organism, *in* Harris, R. S., and Thimann, K. V.: Vitamins and Hormones; Advances in Research and Applications. New York, Academic Press, Inc., 1945, vol. 3, pp. 297–336.
5. Smith, G. V. S., and Smith, O. W.: Internal secretions and toxemia of late pregnancies, *Physiol. Rev.* **28**: 1–22 (Jan.) 1948.
6. Albert, A.: A clinical bio-assay for chorionic gonadotropin, (abstract) *J. Clin. Endocrinol.* **8**: 619–620 (July) 1948.
7. Caton, W. L.; Roby, C. C.; Reid, D. E., and Gibson, J. B.: Plasma volume and extravascular fluid volume during pregnancy and the puerperium, *Am. J. Obst. & Gynec.* **57**: 471–481 (Mar.) 1949.

8. KEATING, F. R., JR.; POWER, M. H.; BERKSON, J., and HAINES, S. F.: The urinary excretion of radioiodine in various thyroid states, *J. Clin. Investigation* **26**: 1138–1151 (Nov.) 1947.
9. KEATING, F. R., JR.; WANG, J. C.; LUELLEN, T. J.; WILLIAMS, M. M. D.; POWER, M. H., and MCCONAHEY, W. M.: The measurement of the iodine-accumulating function of the human thyroid gland, *J. Clin. Investigation* **28**: 217–227 (Mar.) 1949.
10. GASTINEAU, C. F.; ALBERT, A., and RANDALL, L. M.: The renal clearance of chorionic gonadotropic hormone in pregnancy and in neoplasm of the testis, (abstract) *J. Clin. Endocrinol.* **8**: 599–600 (July) 1948.

FERTILITY IN PARAPLEGIC MALES*

A PRELIMINARY REPORT OF ENDOCRINE STUDIES

ERNEST BORS, M.D., EARL T. ENGLE, PH.D., ROBERT C. ROSENQUIST, M.D. AND VICTOR H. HOLLIGER, M.D.

From the Paraplegic Service of Birmingham Veterans Administration Hospital, Van Nuys, California; the Department of Surgery, University of California at Los Angeles School of Medicine; and the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

INTRODUCTION

THE natural desire of patients with spinal cord injuries to have children motivated this study on 34 paraplegic patients with various levels of spinal cord injury. Reproduction among male patients with a spinal cord injury is rare, although not impossible. Recently 2 such cases were reported (1), to which we add 8 of our own, only 1 of which is included in this study. Studies of the sex behavior of paraplegic patients reveal that ejaculations are relatively rare as compared with erections (2, 3, 4), and that the percentage of motile sperm is low in the massaged prostatic contents.

PLAN OF INVESTIGATION AND METHODS

This investigation was planned to obtain data, relevant to the endocrine, genital and neurophysiologic status of the patients. Biopsy specimens were obtained from both testes of all patients reported here. Intravenous sodium pentothal was used for anesthesia in the patients with sensitive gonads.

In all cases an attempt was made to obtain both prostatic and urinary cultures. A history of epididymitis, infections of the upper urinary tract, and urinary calculi was recorded.

Endocrine studies were made in the majority of the cases. The urinary excretion of total neutral, nonphenolic 17-ketosteroids was determined in 33 cases by the method of Robbie and Gibson (5), which is a modification of the Zimmermann method. The alpha and beta fractions were determined by the method of Frame (6). The urinary excretion of estrogens was determined in all 34 cases by the method of Jailer (7). Pituitary gonado-

Received for publication September 29, 1949.

* Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

tropin excretion was determined in 29 cases, using the bio-assay method of Klinefelter, Albright and Griswold (8).

Central thermoregulatory sweating was tested in 29 cases by using dry heat and direct inspection to detect perspiration. Results were recorded on a dermatome chart as published by Keegan (9). A sweat chamber was constructed, with minor modifications, according to Guttmann's (10) recommendations.

The history of sexual behavior and sexual dreams was recorded in all cases.

DATA ON INJURIES

The vertebral level and extent of the injury to the spinal cord are among other data shown in Table 1. There were 6 cervical, 21 thoracic, 6 lumbar and 1 sacral injury. Upon clinical examination 20 were considered complete lesions, and 14 incomplete. Twenty cases were due to fractures caused by accidents and 14 cases occurred consequent to bullet or shell fragment wounds. The shortest time interval between injury and examination was four months, the longest was fifty-six months.

RESULTS OF TESTICULAR BIOPSY

Microscopic examination of all testicular biopsy specimens was made by one of us (ETE), and the pathologic description was written before the clinical status of the patient was known to the examiner.

From these pathologic reports, a grading system based on cell types found in the testis, was set up: No. 1) only Sertoli cells; No. 2) Sertoli cells plus spermatogonia; No. 3) Sertoli cells, spermatogonia and primary spermatocytes; No. 4) Sertoli cells, spermatogonia, primary and secondary spermatocytes; No. 5) Sertoli cells, spermatogonia, primary and secondary spermatocytes and spermatids; No. 6) all cells plus mature sperm, but spermatozoa in reduced numbers; No. 7) normal cell population of a normal testis. This system of grading is admittedly imperfect, and was done from the pathologic descriptions only. As it is an arbitrary classification, it does not permit consideration of the great variation in a group, particularly as regards classes 6 and 7. Class 6 approaches Class 7 (normal), but represents a group of testes which are very poor producers of normal spermatozoa. The correlation between the results obtained with this classification and with the level and extent of the spinal cord injury is demonstrated in Figure 1. This basic curve is used also for the plotting of the results of biopsy against data obtained by sweating tests. There were three cases classified as No. 7, one case of No. 6–7 (the patient's wife conceived and gave birth to a healthy child), ten cases of No. 6, six cases of No. 5–6, one case of No. 5, two cases of No. 4, five cases of No. 3, three cases of No. 2,

TABLE 1

Patient	Age (yrs.)	Date of injury & time between injury and biopsy	Vertebral level of injury & extent of cord lesion	17-Ketosteroids (mg./24 hrs.)	Estrogens (micrograms/24 hrs.)	Gonadotropins (M.U./24 hrs.)	Histology of testicular biopsy specimen	Biopsy grading
E.L.	21	9/28/46 22 mo.	C-5 I*	Alpha 11.9 Beta 1.8 Total 12.7	40	Less than 6.6	Leydig cells normal; secondary atrophy.	6 –
J.C.	35	6/28/47 16 mo.	C-5 I	Alpha 16.5 Beta 1.4 Total 17.9	45.1	More than 6.6 Less than 13.2	Leydig cells normal; right testis normal; left testis showed tubular fibrosis with secondary atrophy.	7
M.G.	27	7/26/44 36 mo.	C-5 C*	Alpha 11.6 Beta 1.9 Total 13.5	92.6	Less than 6.6	Leydig cells small and scattered; secondary atrophy.	1+
W.M.	24	8/24/47 8 mo.	C-6 I	Alpha 13.8 Beta 1.3 Total 15.1	21.6	Less than 52.8 More than 26.4	Leydig cells normal; secondary atrophy.	3
H.K.	23	9/7/47 6 mo.	C-6 I	Alpha 17.7 Beta 5.0 Total 22.7	39.4	Less than 6.6	Leydig cells normal; secondary atrophy.	3
A.V.	37	5/24/47 11 mo.	C-8 I	Alpha 15.8 Beta 1.4 Total 17.2	38.8		Leydig cells normal; secondary atrophy.	2
H.M.	28	10/24/44 41 mo.	D-4 C	Alpha 15.9 Beta 4.1 Total 20.0	51.8	Less than 6.6	Leydig cells normal; secondary atrophy.	2 –
E.S.	21	5/15/45 34 mo.	D-3 I	Alpha 12.1 Beta 1.1 Total 13.2	46.4	More than 13.2 Less than 26.4	Leydig cells normal; secondary atrophy.	1
P.F.	25	5/23/45 34 mo.	D-4 C	Alpha 22.4 Beta 0.5 Total 22.9	70.9	Less than 6.6	Leydig cells normal; tubular fibrosis; secondary atrophy.	6 –
R.L.	31	3/24/45 43 mo.	D-4 C	Alpha 14.3 Beta 1.6 Total 15.9	40	Less than 6.6	In left testis, Leydig cells normal; In right testis, Leydig cells abnormal, showing tubular fibrosis and secondary atrophy.	7
D.S.	23	2/22/45 42 mo.	D-5 C	Alpha 9.8 Beta 0.7	23.2	Less than 6.6	Leydig cells normal; reduced rate of spermatogenesis.	6
R.V.	22	4/26/47 15 mo.	D-5 C	Alpha 14.9 Beta 1.7 Total 16.6	16.9	Less than 6.6	Leydig cells normal; secondary atrophy.	2
C.H.	26	2/6/46 25 mo.	D-7 I	Alpha 9.7 Beta 1.1 Total 10.8	71.2	Less than 6.6	Leydig cells normal; secondary atrophy.	6 –

TABLE 1—*continued*

Patient	Age (yrs.)	Date of injury & time between injury and biopsy	Vertebral level of injury & extent of cord lesion	17-Ketosteroids (mg./24 hrs.)	Estrogens (micrograms/24 hrs.)	Gonadotropins (M.U./24 hrs.	Histology of testicular biopsy specimen	Biopsy grading
J.G.	33	12/20/47 4 mo.	D-10 C	Alpha 14.2 Beta 0.7 Total 14.9	34.6	Less than 6.6	Leydig cells normal; secondary atrophy.	6
H.W.	28	8/9/45 31 mo.	D-10 C	Alpha 19.0 Beta 3.2 Total 22.2	54		Leydig cells sparse and pale; secondary atrophy.	6−
E.A.	30	6/22/47 11 mo.	D-11 I	Alpha 10.8 Beta 1.2 Total 12.0	52.9		Leydig cells normal; secondary atrophy.	3
S.D.	24	4/30/45 39 mo.	D-11 C	Alpha 18.3 Beta 1.2 Total 19.5	30.5	Less than 6.6	Leydig cells: none in the rt. testis; normal in the lt. testis.	7
C.W.	35	7/7/47 12 mo.	D-11 C	Alpha 16.0 Beta 2.5 Total 18.5	41.7	Less than 6.6	Leydig cells normal; tubular fibrosis.	2
A.E.	22	6/6/45 33 mo.	D-11 I	Alpha 16.5 Beta 2.1 Total 18.6	38.5	Less than 6.6	Leydig cells normal; secondary atrophy.	3
E.P.	24	12/6/46 21 mo.	D-11 C		53.4		Leydig cells normal; secondary aplasia of tubules.	3
A.K.	25	3/5/45 32 mo.	D-11 C	Alpha 36.5 Beta 1.4 Total 37.9	42.5	Less than 6.6	Leydig cells normal; tubular fibrosis.	6−
I.L.	56	4/7/46 26 mo.	D-12 C	Alpha 16.3 Beta 1.1 Total 17.4	36.9	Less than 6.6	Leydig cells normal; fibrosis of intertubular area.	4
G.W.	27	3/8/45 42 mo.	D-12 C	Alpha 14.4 Beta 0.7 Total 15.1	34	Less than 6.6	Leydig cells normal; spermatogenic arrest with impaired cell division.	6
D.C.	29	1/23/44 56 mo.	D-12 C	D-12 29.4 Beta 4.0 Total 33.4	32.8	Less than 6.6	Leydig cells normal; atypical cell division with failure to produce mature sperm.	4
T.S.	36	3/13/45 40 mo.	D-12 C	Alpha 6.1 Beta 1.0 Total 7.1	20.3	Less than 6.6	Leydig cells normal; nearly normal testis with incipient progressive tubular fibrosis.	6+
C.R.	28	12/17/44 39 mo.	D-12 C	Alpha 11.5 Beta 1.5 Total 13.4	46.2	Less than 6.6	Leydig cells normal; secondary atrophy.	6
T.G.	29	11/18/44 40 mo.	D-12 C	Alpha 12.1 Beta 1.4 Total 13.5	15.7	Less than 6.6	Leydig cells pale; secondary atrophy.	6

TABLE 1—*continued*

Patient	Age (yrs.)	Date of injury & time between injury and biopsy	Vertebral level of injury & extent of cord lesion	17-Ketosteroids (mg./24 hrs.)	Estrogens (micrograms/24 hrs.)	Gonadotropins (M.U./24 hrs.)	Histology of testicular biopsy specimen	Biopsy grading
T.G.	23	6/5/45 33 mo.	L-1 I	Alpha 15.1 Beta 2.2 Total 17.3	65.9	Less than 6.6	Leydig cells sparse; secondary atrophy.	6
R.A.	29	7/31/43 56 mo.	L-1 I	Alpha 11.4 Beta 0.4 Total 11.8	29.4	Less than 6.6	Leydig cells normal; secondary atrophy.	5
J.M.	28	10/9/44 42 mo.	L-1 C	Alpha 28.1 Beta 1.6 Total 29.7	60.0	More than 6.6 Less than 13.2	Leydig cells normal; tubular fibrosis and secondary atrophy.	6
J.H.	28	6/19/44 45 mo.	L-1 C	Alpha 8.9 Beta 1.0 Total 9.9	0 1.9 (repeat)	Less than 6.6	Leydig cells normal; secondary atrophy.	6
N.K.	45	3/18/46 24 mo.	L-2 I	Alpha 11.3 Beta 1.1 Total 12.4	41.6	Less than 6.6	Leydig cells normal; less atrophy than in most other biopsy specimens.	6
C.H.	39	12/10/46 15 mo.	L-3 I	Alpha 8.4 Beta 0.7 Total 9.1	42	Less than 6.6	Leydig cells normal; severe secondary atrophy.	6−
J.B.	28	6/3/45 33 mo.	S-1 I	Alpha 21.6 Beta 1.3 Total 22.9	17.8		Leydig cells normal; secondary atrophy with reduced rate of spermatogenesis.	6

* I = incomplete transection of the cord. C = Complete transection.

two cases of No. 1–2, and one case of No. 1. Although the three "normal" biopsy specimens were found in patients with injuries at levels of C-5 (incomplete), D-4 (clinically complete) and D-11 (clinically complete), the curve indicates that the lower level injuries were consistently associated with the less damaged testes.

In terms of the usual pathologic classification of Engle (11), the following results were obtained: normal testis—three cases (injury levels at C-5, D-4 and D-11); spermatogenic arrest—two cases (injury levels at C-7 and D-12); tubular fibrosis—seven cases (injury levels from D-4 to L-1); and secondary atrophy—twenty-two cases (injury levels from C-5 to S-1). The classification of secondary atrophy has not previously been used, as this type of histopathology has not been encountered frequently in nonparaplegic cases. Its common histologic features are greatly reduced cell population, a generalized pyknosis of all cells, and complete absence of mitotic activity in any germ cell series.

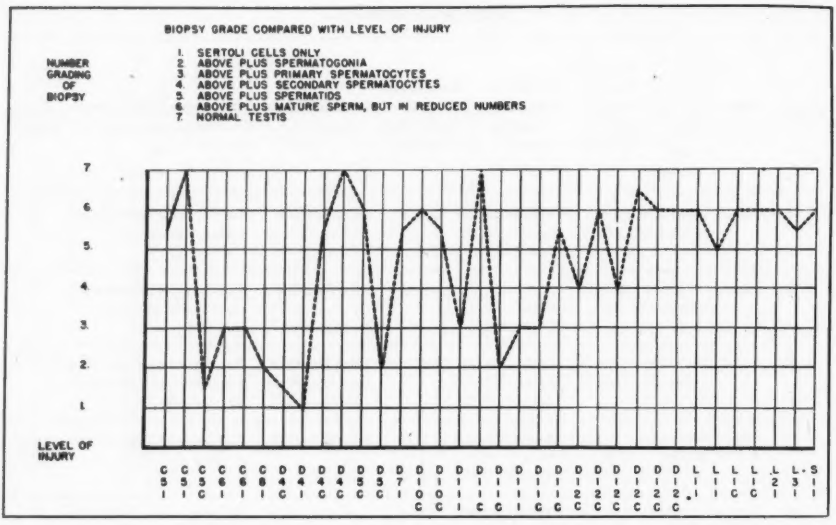

BIOPSY GRADE COMPARED WITH LEVEL OF INJURY
1. SERTOLI CELLS ONLY
2. ABOVE PLUS SPERMATOGONIA
3. ABOVE PLUS PRIMARY SPERMATOCYTES
4. ABOVE PLUS SECONDARY SPERMATOCYTES
5. ABOVE PLUS SPERMATIDS
6. ABOVE PLUS MATURE SPERM, BUT IN REDUCED NUMBERS
7. NORMAL TESTIS
NUMBER GRADING OF BIOPSY
7
6
5
4
3
2
1
LEVEL OF INJURY
C 5 I
C 5 I C
C 5 6 I
C 6 I I
C 6 8 I C
D 4 4 I
D 4 4 C C
D 5 5 C
D 5 7 I
D 10 10 C C
D 11 I
D 11 11 C C
D 11 11 I C
D 11 11 C C
D 11 12 C C
D 11 12 2 C C
D 12 2 2 C C
D 12 2 2 C C C
D 12 2 2 C C C
L 1 1 I
L 1 1 C C
L 1 2 I
L 3 1 I
L-S 1 1

FIG. 1. Comparison between the biopsy grading (ordinate) and the level and extent of the spinal cord injury (abscissa).

C, D, L and S (with numbers from 1 to 12 written vertically underneath) indicate cervical, dorsal, lumbar or sacral vertebral levels, respectively.

C and I, in the lowest (uneven) line throughout the abscissa, indicate clinically complete (C) or incomplete (I) transection of the spinal cord.

The interstitial cells appeared normal in most cases. In some testes these cells were shrunken and fibroblastic. Figures 2 to 4 illustrate the observed histopathology.

Effect of time interval between injury and biopsy

There was no evidence that the time interval between injury and biopsy played a significant role in the testicular pathology. Normal testes were found sixteen, thirty-nine and forty-three months after injury, whereas severe changes occurred as early as six months after injury.

Effect of injury to sympathetic fibers, as determined by sweating tests

Twenty-nine patients underwent sweating tests. This was done in order to determine the level of injury to the sympathetic preganglionic cholinergic fibers. Inasmuch as the gonads are supplied by sympathetics, which control their circulation and therefore their temperature, it appeared desirable to obtain information on at least one of the sympathetic components, namely the cholinergic fibers. It was realized that cholinergic sweating fibers and adrenergic temperature-regulating fibers are not neces-

sarily identical. Although there is agreement that vasoconstrictor and sudomotor fibers leave the cord with the anterior roots, there is still controversy about the vasodilator fibers. These were said to travel over the posterior roots by Foerster (12), whereas Hyndman and Wolkin (13) present evidence that these fibers may leave the cord with the anterior roots. Such considerations seem important in cases of partial injury and for the cautious interpretation of thermoregulatory sweating tests as a means of analysis of damage to the vasomotor fibers of the sympathetics. The results are plotted against the basic curve in Figure 5. In this chart the extent of normal sweating and impaired (*i.e.*, diminished) sweating has been registered, as well as the absence of sweating. In normal sweating there is an abundant outflow of sweat which studs the skin with large pearls and

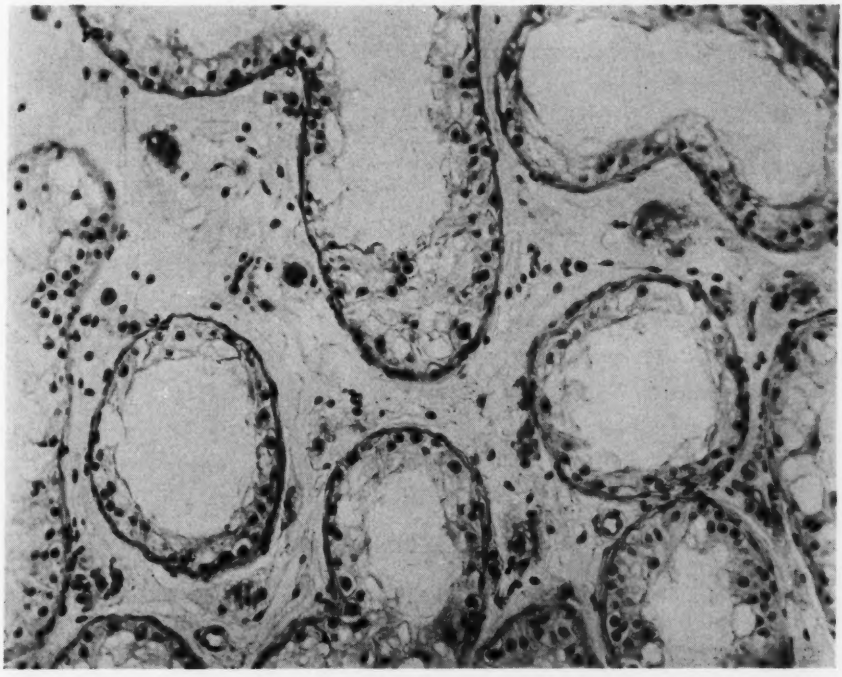


FIG. 2. E. A. (×350) Numerical grading of biopsy—No. 3.

Pathologic report (E.T.E.)

Tubules unequal in size. Tunica propria and basement membrane normal. Cell population severely decreased. Spermatogonia few, 1° spermatocytes more rare, later stages, uncertain. There may be some abortive secondary spermatocytes and spermatids dihisced and free in the lumen.

Intertubular tissue normal.

Diagnosis: Secondary atrophy.

which tends to form a confluent layer; in impaired (diminished) sweating there are only minute droplets confined to the sweat pore; and when sweating is absent the skin remains entirely dry.

There was only one patient with a clinically complete lesion, normal biopsy findings and severely impaired sweating. This contrasts with four patients, graded between No. 3 and No. 4, in whom almost normal sweating was demonstrated. In the remaining patients the extent and degree of

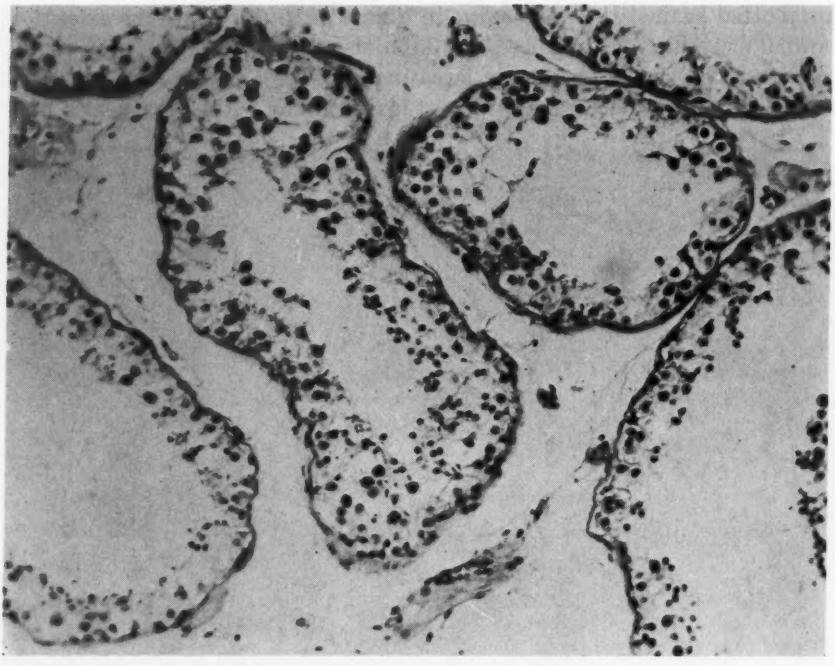


FIG. 3. C. R. (×350) Numerical grading of biopsy—No. 6.
Pathologic Report (E.T.E.)

Tubules of normal size and configuration. Basement membrane and tunica propria not thickened. Within the tubules the Sertoli cell nuclei can be identified, but the cytoplasm of these cells is scanty.

Spermatogonia are located along the basement membrane. They are uniformly poor in cytoplasm. The nuclei are pyknotic. The chromatin network cannot be seen. A few primary spermatocytes are present, with pale nuclei. The chromatin threads are not distinct, as in normal testes. Secondary spermatocytes and spermatids are present in the tubules. Spermatozoa are seen. All of these stages represent perhaps one-tenth of the number of cells found in a normal testis.

The intertubular tissue seems normal. Interstitial cells present, but not abundant Arterioles and capillaries abundant, perhaps dilated.

Diagnosis: Retarded spermatogenesis.

sweating seemed to correspond with the degree of pathology found in the biopsy specimen.

Effect of infections from various sources

A). *Infection of urine and prostate.* There was no apparent relationship between the infection of the urine or prostate, and testicular biopsy findings. All patients with normal biopsy findings (No. 7) showed pathogenic organisms in the cultures of both urine and prostatic fluid, as did the patient classified as No. 6–7, whose wife had a child.

FIG. 4. G. W. ×350) Numerical grading of biopsy—No. 6.
Pathologic Report (E.T.E.)

Tubules normal in size and configuration. Tunica propria and basement membrane normal. Spermatogonia present in normal frequency and with normal nuclear character. Sertoli cells normal. Primary spermatocytes are most numerous. Mitotic spindles are seen (usually rare in normal testis), many with chromatin in loose spireme, and many with clumped and club-like chromatin threads. Many spermatocytes 1° are dehisced and are free in the lumen.

Secondary spermatocytes are rare in most tubules. Many are binucleate. Spermatids and sperm heads are rare. Sperm heads are free and not anchored to Sertoli cells.

Intertubular tissue and arterioles normal.

Diagnosis: Spermatogenic arrest with impaired cell division.

Urinary cultures revealed various degrees of infection. The organism most frequently encountered was *A. aerogenes;* then followed *B. proteus, Staph. aur. non-hem., Streptococcus, E. coli, Ps. aeruginosa, Staph. aur. hem.*

Of the prostatic cultures, all but two showed bacterial growth. In seven cases prostatic fluid for culture could not be obtained although the pros-

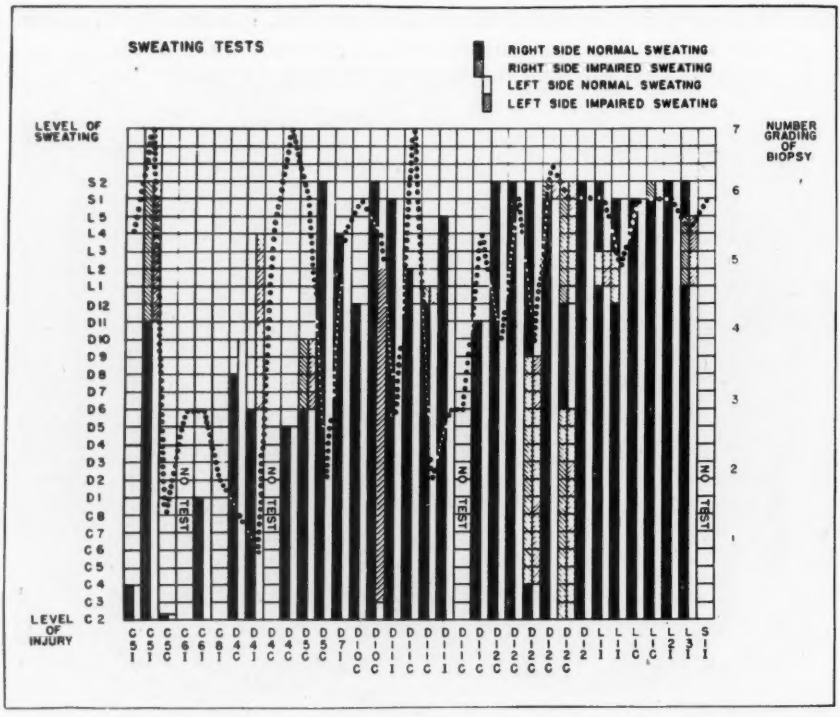


FIG. 5. Comparison between the biopsy grading (broken line) and the extent of sweating (columns) as related to the individual case. Blank spaces indicate absence of sweating. The level and extent of spinal cord injury are recorded in the abscissa (for key, *see* legend to Fig. 1). The ordinate on the left is used to indicate the level of sweating, whereas the ordinate on the right serves to plot the results of biopsy grading.

tate was massaged on three different occasions. The organisms encountered, according to their frequency, were *Staph. aur. non-hem., A. aerogenes* and *B. proteus.*

There were only four cases in which the flora of the prostatic fluid and of the urine were identical. In five cases, one of the various species present in the urine was also obtained from the prostate; but in the remaining cases

the findings in the urine and in the prostate differed completely. There were four patients with suprapubic and two with intraurethral catheters. The remaining patients had a "balanced cord" bladder.

B). *Epididymitis.* There was a history of unilateral, bilateral or unilateral recurrent epididymitis in ten cases. The distribution, according to our testicular biopsy classification, was: two cases in grades No. 1–2, one case in No. 4, two cases in Nos. 5–6, four cases in No. 6, and one case in grade No. 7 (on the contralateral side). According to Engle's (11) classification, six cases showed secondary atrophy, one case spermatogenic arrest, and three cases tubular fibrosis. Three cases revealed a combination of secondary atrophy with some tubular fibrosis. With the exception of one patient who had a complete fibrotic testis secondary to epididymitis and orchitis, with a normal contralateral testis, no direct relationship between the episodes of epididymitis and the biopsy findings could be demonstrated.

C). *History of repeated acute upper urinary tract infection.* Chills and fever due to urinary infections were recorded in twenty-four cases, but ten patients never had such an episode. The frequency of these attacks varied from one to fourteen. There was one case with almost continuously recurring bouts due to bilateral renal calculi with infection. It is this patient who became a father despite the frequent episodes of chills and fever. No correlation could be demonstrated between the recurrent attacks and the biopsy findings, inasmuch as the three patients with normal testes had a history of such attacks in the past, whereas patients without a history of urinary flareups had definitely impaired testes.

D). *Urinary calculosis.* There were eighteen cases with vesical calculi, seven cases with renal calculi (among them one case with bilateral renal calculi) and two cases with a combination of vesical and renal calculi. None of the three patients with a grading of No. 7 had renal calculi but one had bladder calculi. The patient whose marriage was fertile had bilateral renal calculi. No direct correlation between calculosis and biopsy findings could be established.

E). *Incidence of decubitus ulcer.* Twenty patients had had one or more decubitus ulcers. One of the patients had ten decubitus ulcers upon return from overseas. He had normal biopsy findings three years after injury. There is no evidence of a direct relationship between the presence of even multiple decubitus ulcers and the degree of testicular damage.

F). *Miscellaneous sources of infection.* In addition to the decubitus ulcers five patients had a history of pneumonia, recurrent pneumonia, pleurisy, empyema and jaundice respectively in the post-traumatic period. No relationship between these diseases and the biopsy findings could be established.

Influence of hormonal level

A). *17-Ketosteroids.* A determination of the urinary excretion of 17-ketosteroids in twenty-four hours was done in thirty-three cases. A range from 7.0 to 18.0 mg. was accepted as normal. Among paraplegics these levels ranged from 15.0 to 26.0. Only one case showed a subnormal level. Twenty-three patients had levels within the normal range, of which six were at the upper limit of normal, and nine were above the upper limit of normal. There was no obvious correlation between the biopsy findings and the excretion of 17-ketosteroids. Furthermore, no evidence was found of a correlation between the level and extent of the cord lesion and 17-ketosteroid excretion level.

B). *Estrogen excretion.* Urinary estrogen determinations were made in all cases. The normal range was from 6 to 20 micrograms in twenty-four hours. In only one case was the level below normal, in three cases it was within normal range, and in thirty cases it exceeded the upper limit of normal. The patients with normal biopsy findings were among those with elevated estrogen levels. No correlation was found between the biopsy findings and the urinary estrogen level. There was also no correlation between the level and extent of the cord injury and the excretion of estrogen.

C). *Pituitary gonadotropins.* Bio-assays of urinary gonadotropins were carried out in twenty-nine patients. The lowest level tested was 6.6 mouse units. A range of from 0 to 52 mouse units within twenty-four hours was accepted as normal on the basis of control assays run on normal individuals of proven fertility. In only one patient was gonadotropin excretion at the upper limit of normal, in three patients it was well within the normal range and in the rest it was at the lower limit of normal. No correlation was demonstrated between the biopsy findings and the gonadotropin excretion. There was also no correlation between the level and extent of the cord injury and the urinary gonadotropins.

Influence of sex function

A). *Erection and ejaculation.* Of the thirty-four patients, thirty had a return of erections after the injury and four did not. These four men had cord lesions below D-7. Of the thirty patients who had a return of erections, four lost this function subsequent to subarachnoid alcohol block. This leaves twenty-six patients with capability for erections. Among these twenty-six men, eighteen had complete and eight had incomplete erections. Five patients regained the ability to ejaculate. There was no evidence of a relationship between the testicular biopsy findings and sex function. Among eighteen patients with complete erections, sixteen had testes showing normal interstitial cells and two had testes with sparse and pale Leydig cells. Eight

patients had incomplete erections. In five of these, there were normal Leydig cells in the testis; but in three cases the cells were found to be sparse, or pale, or abnormally pigmented or fibrotic. Eight patients had no erections. Of these, seven had normal interstitial cells in the testis and one had scattered small cells. Four men in this last group had a previous subarachnoid alcohol block for the treatment of skeletal muscular spasticity, subsequent to which erections, present prior to the block, disappeared. These observations seem to indicate that interstitial cells do not play an important role in the return of erections.

B). *Sexual dreams.* Ten patients did not recall any sexual dreams. Fourteen patients remembered incomplete dreams and ten patients, complete dry dreams. (An incomplete dream lacks orgasm; a complete dry dream contains orgasm but lacks ejaculation; and a complete wet dream contains both orgasm and actual seminal emission.) There was no correlation between either the incidence or type of dream and biopsy findings. Neither was there a correlation between the incidence or type of dream and the level or extent of the cord injury.

DISCUSSION

The superiority of testicular biopsy as a method for the determination of fertility is well known. Engle (11), Hotchkiss (14), Charny and Moranze (15), (16), and Michelson and Michelson (17) have discussed this fact in papers which do not deal with paraplegics but which correlate ejaculate and biopsy findings. Biopsy was used in seven of eighteen paraplegic cases, studied by Horne, Paull and Munro (1). Upon microscopic examination, these authors found the testis to be normal in three cases, and in the remaining cases to show either an absence of spermatogenic cells, spermatogenic arrest, or tubular atrophy, combined with hyperplasia of the Leydig cells. In our series no hyperplasia of the Leydig cells was encountered. There was one instance of vascular pathology. This patient, with an incomplete cervical lesion, did not show other evidence suggestive of generalized peripheral vascular disease.

The uniformity of the biopsy findings in the group with secondary testicular atrophy was so striking that upon studying the histologic sections one (ETE) wondered whether or not all of them might not have come from one patient. The only difference was in the degree of atrophy. In general, the cells were pyknotic, there was no mitotic activity, and the number of cells at each stage of spermatogenesis was greatly reduced below normal. These findings, not dissimilar to those in the testes of hibernating animals with seasonal cryptorchid testes, suggested the possibility of secondary atrophy, on a neurologic basis, due to hyperemia and hyperthermia. Since both the testis and its blood vessels are innervated by sympathetics (18), sweating

tests should reveal the presence or absence of at least the cholinergic component of this system. These tests confirmed, with a few unexplained exceptions, that the extent of sweating coincided with the more nearly normal testicular structure found in patients with injuries at and below D-11. This was in contrast to the definitely impaired testicular structure of patients with impaired sweating and lesions above D-11 (Fig. 5). Actual temperature studies of the testis will yet be necessary in order to determine the possible role played by the adrenergic part of the sympathetic nerve supply, responsible for the circulatory regulation of the organ. Observations on female paraplegics seem to support the hyperthermia theory for the male, because no change of the menstrual cycle has been noted in the few female paraplegics known to us. Compared to the male gonads, the female gonads are constantly exposed to a higher intraperitoneal temperature.

Mitchell's (18) dissections of the testicular nerve supply demonstrate that fibers from the renal ganglia and intermesenteric nerves reach the testis. There is no evidence as to the level at which these pathways leave the spinal cord. This supply may originate anywhere in the thoracolumbar sympathetic outflow from D-3 to L-2. According to Pick and Sheehan (19) the splanchnic nerve originates most frequently at D-6, putting the upper border of the outflow at that level. Although there is no evidence that testicular thermoregulatory efferent fibers, and pain-conducting afferent fibers are localized at the same segments in the cord, a study now in progress (EB) has revealed that testicular pain produced by squeezing is not elicited in patients with anesthesia at the D-9 and D-10 dermatomes. It is an interesting coincidence that the biopsy grading improves below this vertebral level. No conclusions are drawn from this observation because of one patient in whom the histologic sections showed a normal testis, despite the absence of testicular sensation.

In our experience a contracted scrotum can be observed at times in paraplegic patients with upper motor neuron lesions and a tendency toward spasticity. Moreover, the cremaster muscle pulls up the testes during these periods of spasticity. This may be an additional factor precipitating testicular changes, since the thermoregulatory role of the tunica dartos (14) is well known. Testicular atrophy has been observed by one of us (ETE) in a nonparaplegic individual with a shortened spermatic cord. The effect of hyperthermia on the germinal epithelium is shown in the absence of spermatogenesis in cryptorchidism, or the reduction of the sperm count demonstrated by McLeod and Hotchkiss (20) after fever therapy. A reduction of fertility was reported by H. Chapman Pincher (21) to occur in white men in the tropics, where even among the natives it is said that more children are conceived during the cooler months. In this connection it appears worth while to report the experience of one patient. This 30-year-old man with an injury at the vertebral level D-12 and at the dermatome

levels of L-3 left and D-10 right, had a biopsy grading of No. 4. On prostatic massage, an initial count of 600,000 immotile sperm per cubic centimeter of semen was found. After application of ice bags two to three times a day for thirty minutes, his sperm count increased. After three months, a sperm count of 33 million/cc., with 4 per cent motile forms, was observed.

If one reviews the incidence of pregnancy in the patients' wives, from the reports of Horne, Paull and Munro (1) and from our own cases, it is observed that pregnancy occurred in the wives of ten patients. Two of these patients had clinically complete cord lesions, one at D-4 (our observation, not discussed in this study) and the other at D-6 (Horne, Paull and Munro). The remaining cases consisted of one partial C-5 lesion (Horne, Paull and Munro), and seven partial thoracic or lumbar lesions (of which only one patient (N.K.) with an incomplete lesion at L-2 appears in this study). Obviously, when the sympathetic nerves are not impaired below D-4 or D-6, but only released from cerebral control, ejaculation is not necessarily abolished. However, the coordination between emission (sympathetic) and ejaculation (somatic) is frequently disturbed. Therefore, ejaculation is rarely encountered in patients with high lesions (3, 2, 4). The greater incidence of conception in the wives of individuals with low, and especially with partial lesions, seems more understandable when one considers the lesser damage to the testes and the less frequently impaired ejaculation mechanism in this group of patients.

So far, only a neurogenic hypothesis has been presented as a possible explanation for our observations. It is felt that a hormonal imbalance may also be a significant factor in producing tubular atrophy in many of these patients. In brief, the endocrine findings are as follows: elevated urinary 17-ketosteroid levels in nine of thirty-three cases (with six others at the upper limit of normal), elevated urinary estrogen levels in thirty of thirty-four cases, and low urinary gonadotropin levels (in spite of severe testicular atrophy) in twenty-five of twenty-nine cases. Three-eighths of the 17-ketosteroids are said to originate from the interstitial cells of the testis and five-eighths from the adrenal cortex (22). Biopsies showed no evidence of Leydig cell hyperplasia. Estrogens (23) are secreted by the adrenal cortex in the male, Therefore, the high excretion values obtained must be due either to overproduction of estrogens by the adrenal cortex or impaired destruction of estrogens due to liver disease. Hepatic function tests (cephalin-flocculin, thymol turbidity, and quantitative Van den Bergh) have failed to reveal any significant degree of liver disease in our patients. A quadriplegic patient excreting 92.6 micrograms of estrogen per twenty-four hours, had normal hepatic function as measured by these tests.

In an attempt to correlate the endocrine findings and the impairment of fertility, the possibility of adrenal hyperactivity has been considered. The paraplegic is subjected to numerous alarming stimuli such as urinary tract

infection, decubitus ulcers or spinal shock, which may well give rise to adrenal cortical hyperactivity by means of the adaptation mechanism described by Selye (24). The elevation of the 17-ketosteroid and estrogen excretion levels (without evidence of impaired hepatic function) is suggestive of hyperfunction of the adrenal cortex, although it is recognized that the Sertoli cells of the testis may also produce estrogen (25). Gamma globulin determinations on serum (26) gave normal values in thirteen of the nineteen cases in which this test was made: in the six remaining cases, four patients had elevated levels and two patients had subnormal levels.

Adrenal cortical hyperfunction may impair fertility by (a) indirect alteration of the pituitary-gonadal axis, or (b) direct action of androgens on germinal epithelium (27). In the cases under study, gonadotropin secretion may have been inhibited by the high level of estrogen in addition to androgens; either can inhibit secretion of gonadotropins. In addition estrogens have proven to be inhibitory for spermatogenesis (28). Thus, although severe atrophy of the germinal epithelium was present in our patients, high titers of gonadotropins were not found.

In the study of infertility in paraplegics, examination of the ejaculate is far inferior to biopsy because: 1) rarely can an ejaculate be obtained; 2) specimens obtained by prostatic massage reflect more the degree of infection of the adnexa than the condition of the testis. The seminal vesicles are not a storage room for sperm, as has been recently stated (1). The function of the seminal vesicles, *i.e.*, the secretion of fructose and ascorbic acid, has been described by Huggins (29). The amount of the prostatic specimen is said by Horne, Paull and Munro to be increased by prior electric rectal stimulation (1). Although we followed accurately the technique given by these authors, we have not been impressed by the results. This method has been used in animal experiments by Durfee, Lerner and Kaplan (30) and in man by Joel (31). It was not applied by these authors to patients with spinal cord lesions.

Although it is known that febrile disease has an adverse effect on spermatogenesis, no direct correlation between the condition of the testis and renal infection, calculosis, decubitus ulcer or other sources of infection could be established. This is probably due to the fact that the infection would have only a temporary effect on the spermatogenesis. The indirect endocrinologic effect of these infections has been discussed in the preceding paragraphs. In this connection it is of interest to mention that Huggins (29) does not consider that there is a correlation between infection of the prostate and fertility.

SUMMARY

1. A study of the fertility of 34 men with spinal cord injuries is presented.

2. Microscopic examination of testicular biopsy specimens revealed pathology in all but 3 cases. The predominant picture was tubular atrophy and no disturbance of Leydig cells.

3. With 2 exceptions, there seemed to be a correlation between the testicular biopsy findings and the level of the spinal cord lesion; a lesser degree of testicular pathology was encountered when the cord lesion was at a level below D-11.

4. Testicular biopsy findings corresponded to the results of sweating tests, with 4 exceptions. The association of lesser testicular pathology with normal sweating and major testicular pathology with impaired sweating, indicates a direct relationship between testicular function and the autonomic nervous system.

5. There was no correlation between the biopsy findings and sexual behavior.

6. Urinary 17-ketosteroid levels were at the upper limit of normal or were elevated, in 15 of 33 determinations.

7. Urinary estrogen levels were elevated in 30 of the 34 patients.

8. Urinary gonadotropins, determined in 29 cases, were low in 25 and normal in 4.

Acknowledgment

We want to express our appreciation to those who actively contributed in this investigation. They are: Dr. Roy H. Nyquist who performed the sweating tests; the Medical Illustration and Photography Department of Birmingham Hospital; and Mr. Herbert O. Carne, Miss Ellen Bahr and Mrs. Virginia Crystal, who helped with the chemical examinations and bio-assays.

REFERENCES

1. Horne, H. W. Jr.; Paull, D. P., and Munro, D.: Fertility studies in the human male with traumatic injuries of the spinal cord and cauda equina, *New England J. Med.* **239**: 959–961, 1948.
2. Bors, E.: Spinal Cord Injuries. Veterans Administration Technical Bulletin, TB10-503, 1948, pp. 26–27.
3. Munro, D.; Horne, H. W., Jr., and Paull, D. P.: The effect of injury to the spinal cord end cauda equina on the sexual potency of men, *New England J. Med.* **239**: 903–911, 1948.
4. Talbot, H. S.: A report on sexual function in paraplegics, *J. Urol.* **61**: 265–270, 1949.
5. Robbie, W. A., and Gibson, R. B.: Rapid clinical determination of urinary 17-ketosteroids, *J. Clin. Endocrinol.* **3**: 200–205, 1943.
6. Frame, E. G.: A micro method for the separation of 17-ketosteroids into alpha and beta fractions, *Endocrinology* **34**: 175–180, 1944.
7. Jailer, J. W.: A fluorometric method for the clinical determination of estrone and estradiol, *J. Clin. Endocrinol.* **8**: 564–579, 1948.
8. Klinefelter, H. F., Jr.; Albright, F., and Griswold, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3**: 529–544, 1943.

9. KEEGAN, J. J.: Dermatome hypalgesia with posterolateral herniation of lower cervical intervertebral disc, *J. Neurosurg.* **4:** 115–139, 1947.
10. GUTTMANN, L.: The management of the quinizarin sweat test, *Post-Grad. M. J.* **23:** 353–366, 1947.
11. ENGLE, E. T.: The testis biopsy in infertility, *J. Urol.* **57:** 789–798, 1947.
12. FOERSTER, O.: The dermatomes in man, *Brain* **56:** 1–39, 1933.
13. HYNDMAN, O. R., and WOLKIN, J.: The autonomic mechanism of heat conservation and dissipation. I. Effects of heating the body; evidence for existence of capillary dilator nerves in anterior roots, *Am. Heart J.* **22:** 289–304, 1941.
14. HOTCHKISS, R. S.: Fertility in Men. Philadelphia, J. B. Lippincott Co., 1944.
15. CHARNY, C. W., and MORANZE, D. R.: Testicular biopsy. Further studies in male infertility, *Surg., Gynec. & Obst.* **74:** 836–842, 1942.
16. CHARNY, C. W.: Testicular biopsy: its value in male fertility, *J.A.M.A.* **115:** 1429–1433, 1940.
17. MICHELSON, L., and MICHELSON, R.: Fertility studies of the male in barren marriages, *J.A.M.A.* **134:** 941–943, 1949.
18. MITCHELL, G. A. G.: The innervation of the kidney, ureter, testicle and epididymis, *J. Anat.* **70:** 10–32, 1935.
19. PICK, J., and SHEEHAN, D.: Sympathetic rami in man, *J. Anat.* **80:** 12–20, 1946.
20. MACLEOD, J., and HOTCHKISS, R. S.: The effect of hyperpyrexia upon spermatozoa counts in men, *Endocrinology* **28:** 780–784, 1941.
21. PINCHER, H. C.: Effect of temperature on fertility of the male, *Nature, London* **155:** 272 (March 3) 1945.
22. HELLER, C. G., and NELSON, W. O.: Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis and treatment, *J. Clin. Endocrinol.* **8:** 345–366, 1948.
23. SOFFER, L. J.: Clinical and experimental studies of adrenal cortical hyperfunction, *Bull. New York Acad. Med.* **24:** 32–50, 1948.
24. SELYE, H.: The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol.* **6:** 117–230, 1946.
25. BERTHRONG, M.; GOODWIN, W. E., and SCOTT, W. W.: Estrogen production by the testis, *J. Clin. Endocrinol.* **9:** 579–592, 1949.
26. KUNKEL, H. G.; AHRENS, E. H., JR., and EISENMENGER, W. J.: Application of turbidimetric methods for estimation of gamma globulin and total lipid to the study of patients with liver disease, *Gastroenterology* **11:** 499–507, 1948.
27. PULLEN, R. L.; WILSON, J. A.; HAMBLEN, E. C., and CUYLER, W. K.: Clinical reviews in andrologic endocrinology. III. Treatment of seminal failure, *J. Clin. Endocrinol.* **2:** 730–736, 1942.
28. MCDONALD, D.: *in* Conference on Diagnosis in Fertility, edited by Earl T. Engle. Springfield, Illinois, Charles C Thomas. Publisher, 1946.
29. HUGGINS, C.: *in* Conference on Diagnosis in Fertility, edited by Earl T. Engle. Springfield, Illinois, Charles C Thomas. Publisher, 1946.
30. DURFEE, T.; LERNER, M. W., and KAPLAN, N.: The artificial production of seminal ejaculation, *Anat. Rec.* **76:** 65–68, 1940.
31. JOEL, C. A.: Über eine Modifikation des elektrischen Ejakulationstestes, *Schweiz. med. Wchnschr.* **71:** 1074–1075, 1941.

HYPOKALIEMIC, HYPOCHLOREMIC ALKALOSIS IN CUSHING'S SYNDROME. OBSERVATIONS ON THE EFFECTS OF TREATMENT WITH POTASSIUM CHLORIDE AND TESTOSTERONE*

ROBERT TEABEAUT, M.D., FRANK L. ENGEL, M.D. AND HAYWOOD TAYLOR, PH.D.

From the Departments of Medicine and Biochemistry, Duke University School of Medicine, Durham, N. C.

ALKALOSIS associated with hypokaliemia and hypochloremia in certain cases of Cushing's syndrome has been reported by McQuarrie *et al.* (1), and Anderson *et al.* (2) and studied in detail by Kepler and his associates (3, 4, 5, 6, 7). Although the precise mechanism responsible for this disturbance in electrolyte balance has not yet been elucidated, a basis for an understanding of it has come from the studies of Darrow (8, 9). This investigator has shown that, in rats at least, there is a high degree of correlation between the sodium and potassium content of muscle and the serum chloride and bicarbonate after biologic equilibrium has been established. Deficit of potassium induced by a low potassium diet and/or injections of desoxycorticosterone resulted in an increase in serum bicarbonate and muscle sodium and a decrease in muscle potassium and serum chloride. Similar changes were found during alkalosis from chloride deficit. Rats suffering from acidosis due to sodium deficit exhibited a decreased intracellular sodium with normal or slightly elevated muscle potassium when serum bicarbonate was decreased.

Based on the experimental studies of Darrow on the effects of desoxycorticosterone acetate on muscle composition, Kepler *et al.* (6) have investigated the muscle composition in several cases of Cushing's syndrome and have confirmed the fact that in the presence of alkalosis with hypochloremia and hypokaliemia there is a deficit in intracellular muscle potassium, the latter being replaced by sodium. This finding, together with the other observations by this same group of investigators that the alkalosis of Cushing's syndrome is not amenable to treatment with ammonium chloride, that it is made worse by restriction of potassium in the diet and is alleviated by ingestion of extra amounts of potassium, all support the view that an intracellular deficit of potassium may be of prime importance in

Received for publication October 1, 1949.

* Presented at the Thirty-first Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 3, 1949.

the production of alkalosis in this syndrome. The precise mechanism of the deficit in potassium is still obscure, the negative potassium balance being out of proportion to the negative nitrogen balance.

The purpose of this report is to describe an additional case of Cushing's syndrome with alkalosis in which observations were made on the effects of a single massive intravenous dose of potassium chloride and of treatment with testosterone propionate.[1] It was felt that studies with this hormone might throw further light on the mechanisms involved in the alkalosis, since testosterone propionate has been reported to induce a positive potassium balance with retention of potassium intracellularly even at the expense of extracellular potassium depletion (10). An extreme lowering of serum potassium may occur in the face of a decreased renal excretion of potassium in some individuals treated with this hormone.

REPORT OF CASE

A 34-year-old single white male farmer was admitted to Duke Hospital on January 18, 1949, and discharged five weeks later. Family and past histories were noncontributory. During the two years before admission polyphagia, polydypsia, and polyuria developed. He gained weight rapidly from an average of 144 lbs. to 204 lbs. and multiple furuncles appeared on his arms, neck, trunk, and lower extremities. The furuncles were treated with antibiotics and incisions and drainage but there were repeated recurrences. With the increase in weight, which was largely distributed about the trunk, he noted fullness of his face with a reddish discoloration of his normally fair skin, a dusky color of his extremities, purple striae on his abdomen and a tendency to bruise easily. In January 1948, diabetes mellitus was diagnosed and treated with diet and 40 units of protamine zinc insulin daily. Progressive weight loss began about this time and amounted to 75 pounds by the time of admission. Four months before admission hypertension was first noted. Back pain, especially in the costovertebral angle, had been progressive for two years and this, in addition to weakness and progressive disability, confined him to bed for four months prior to admission.

Physical examination revealed a chronically ill, weak male who obviously had lost considerable weight, particularly in the extremities, yet had a full, plethoric, moon-shaped face and a shoulder fat pad (Fig. 1B). Petechiae, ecchymoses and scars from previous furuncles were scattered over the entire body. Purple striae over the abdomen and legs were prominent. The forehead hair line had receded and face hair was sparse although trunk hair growth was ample. There was slight exophthalmos, tortuosity of retinal vessels, but no visual field defects. The thyroid gland was not enlarged and the thorax and abdomen were normal, save for changes noted above. The blood pressure was 190/110. The genitalia and rectum were normal. There was a marked kyphosis and tenderness, especially on the right in the regions of T_{12} and L_1.

The essential laboratory findings are as follows: Blood morphology: R.B.C. 4.4 million, hematocrit 42 per cent, reticulocytes 2.3 per cent, W.B.C. 13,250 (seg. 93, mono. 2, lym. 5 per cent). Blood chemistry: sugar 85 mg. per cent, NPN. 30 mg. per cent,

[1] The testosterone propionate used in this study was supplied by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products.

sodium 152 mEq./L., chloride 82 mEq./L., CO_2 combining power 41 mEq./L., potassium 1.8 mEq/L., total protein 5.9 Gm. per cent, albumin 3.1 Gm. per cent, globulin 2.8 Gm. per cent, bilirubin 0.5 mg. per cent, calcium 8.5 mg. per cent, phosphorus 2.4 mg. per cent, alkaline phosphatase 4.5 Bodansky units, cholesterol 280 mg. per cent. During a glucose tolerance test the fasting blood sugar was 141 mg. per cent; ½ hr., 185 mg. per cent; 1 hr., 235 mg. per cent; 2 hr., 265 mg. per cent; and 3 hr., 210 mg. per cent. The bromsulfalein test for liver function showed 3 per cent retention in forty-five minutes; prothrombin time normal; thymol turbidity 0.5 units; flocculation 0. The serologic

A

B

FIG. 1. A 34-year-old man with Cushing's syndrome (B). The photograph above (A) was taken three years prior to the onset of the disease.

test for syphilis gave negative results. Urine: S.G. 1.007–1.020; albumin, a trace; W.B.C., 8–15 per h.p.f.; culture showed *Staph. aur. hem.*; reducing steroids, 7.7 mg. per twenty-four hours; 17-ketosteroids, 12.1 mg. per twenty-four hours. The basal metabolic rate was minus 15 per cent. X-ray examination of the chest showed an elevation of the right diaphragm and densities in the right lower and upper lung fields, which had the appearance of metastatic lesions. There was marked osteoporosis of the vertebral bodies, with collapse of T_{12} anteriorly.

The patient's course was one of gradual deterioration with increase in size of pulmonary lesions, development of pleural effusions in the right chest and loss of urinary bladder control and other signs of cord compression. He was never in good enough condition to permit surgical exploration. The patient died six weeks following discharge. No necropsy was obtained.

METHODS

During the period of study, except where indicated, the patient received a weighed diet containing 2,340 calories (carbohydrate 250 grams, protein 110 grams and fat 100 grams) and was given 35 units of protamine zinc insulin daily. Regrettably, no facilities were available for carrying out a proper balance study so data are available only on the calculated intake and on the urinary output of electrolytes for a brief period during and following the infusion of potassium chloride.

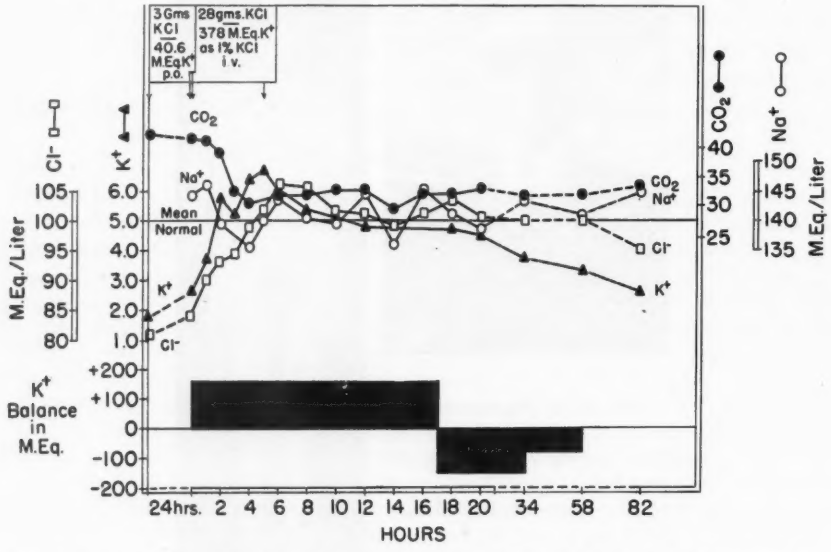


FIG. 2. Serum electrolyte changes and potassium balances during and after the intravenous infusion of 28 grams of potassium chloride.

All chemical determinations were carried out in the clinical laboratory of the department of Biological Chemistry. The plasma carbon dioxide combining power was determined by the open method of Van Slyke (11), the serum and urine sodium and potassium by a Barnes flame photometer (12) and serum chloride by the method of Whitehorn (13).

RESULTS

Previous studies have indicated the efficacy of the ingestion of potassium salts in the correction of alkalosis in this syndrome (4, 5). In this study it was decided to investigate the effects of a single massive intravenous injection of potassium chloride on the serum electrolyte and electrocardio-

graphic abnormalities. The patient was fasted overnight. Protamine zinc insulin was omitted on the morning of the study and the patient received no food until 6 P.M. on that day. During and after the infusion of potassium chloride, serum electrolytes were determined every hour for the first six hours and thereafter every two hours until the twentieth hour. Electrocardiographic tracings were made every thirty minutes during the infusion and at frequent intervals thereafter. Urine was collected during the day of infusion and for the following thirty-six hours and analyzed for potassium. Potassium balances were calculated from the potassium excretion and the intake as infusion plus the calculated intake in the one meal on the day of

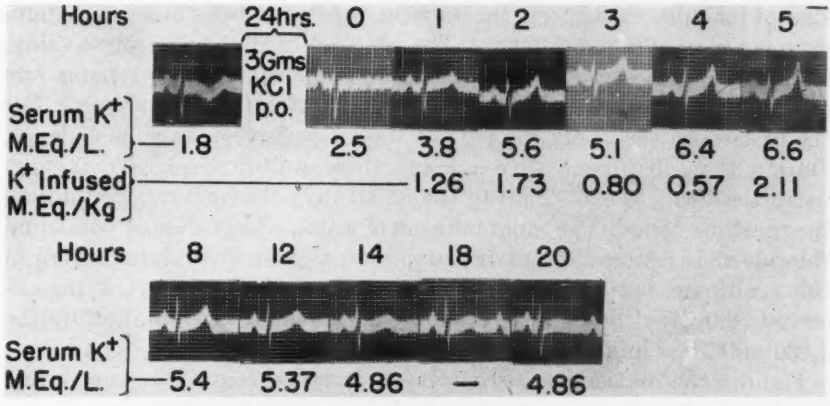


FIG. 3. Electrocardiographic pattern (Lead II) before, during and after infusion of 28 grams of potassium chloride. The amount of K^+ infused per hour per kilogram of body weight is recorded beneath the serum potassium levels in the upper column.

the test and the meals during the following thirty-six hours. Since the calculated potassium content of the diet, which was not reweighed after the patient ate, is at best only an approximation it is clear that the balance figures to be reported here are only crude estimates. No account was taken of the potassium ingested on the day prior to the test.

Figure 2 shows the results of this study. On the day before the infusion the patient had been given 3 grams of potassium chloride by mouth. As a result, a rise from the very low levels of serum K^+ and Cl^- and a small decrease in the serum CO_2 combining power took place. The potassium chloride was infused as a 1 per cent solution over a five-hour period, being discontinued when the electrocardiogram had shown a definite elevation of the T-waves (Fig. 3) and the serum potassium had increased to 6.6 milliequivalents per liter after 28 grams of potassium chloride had been in-

fused. Note that as the serum potassium and chloride concentrations increased rapidly during infusion of KCl, the CO_2 combining power and sodium concentration of the serum decreased towards normal, The serum potassium reached its peak at the end of the infusion. and then declined to a normal level during the next fifteen hours. Thereafter it decreased steadily, being 2.8 milliequivalents per liter sixty hours later. The serum chloride remained essentially normal after the infusion until the final determination, when a definite decrease was noted. The CO_2 combining power reached a minimum of 31 milliequivalents per liter, a level still somewhat above normal. Thereafter it rose to, and remained at, definitely abnormal levels, although these values were not as high as those prior to treatment. Except for a decrease during the infusion, the fluctuations in serum sodium were not particularly meaningful. The approach of all the electrolyte values toward the mean normal line during and immediately after potassium chloride infusion and their deviation from it thereafter is impressive. For the first seventeen hours the patient was in positive potassium balance. During the subsequent sixteen hours there was a negative potassium balance which was equivalent to almost all the potassium retained during the previous period. The rapid infusion of a single large dose of potassium chloride thus restored to nearly normal the altered electrolyte pattern in this syndrome, but the improvement was only transient. Part of the observed changes also might be attributed to the effects of dilution by the 2,800 milliliters infused.

Figure 3 records the electrocardiographic changes (lead II) before, during and for twenty hours after the infusion of KCl. Prior to therapy, and at a time when the serum potassium was only 1.8 milliequivalents per liter, the electrocardiogram showed the characteristic findings associated with hypokaliemia, *i.e.*, a prolonged Q-T interval and an inverted T-wave. Twenty-four hours after the ingestion of 3 grams of potassium chloride there was a rise in the serum potassium to 2.5 milliequivalents per liter and a change in the electrocardiographic tracing, the T-wave becoming isoelectric. During the infusion of potassium chloride there was a rapid change in the electrocardiographic pattern, the Q-T intervals becoming shorter and the T-waves becoming high and peaked. This was most striking when the serum potassium had reached a level of 6.6 milliequivalents per liter. During the hour in which this peak was reached, K^+ (2.11 milliequivalents per kilogram body weight) was infused. After discontinuing the infusion the height of the T-wave gradually decreased, becoming isoelectric at the twentieth hour, although the serum potassium was still normal, *i.e.*, 4.86 milliequivalents per liter. Since the Q-T interval was normal at this time it is altogether likely that this T-wave change was related to

hypertensive cardiovascular disease rather than being indicative of potassium deficiency.

Figure 4 presents the results of treatment with potassium chloride by mouth and testosterone propionate intramuscularly, while the patient had a constant dietary intake of potassium. At the beginning of the study the serum electrolytes and the electrocardiogram showed the abnormalities

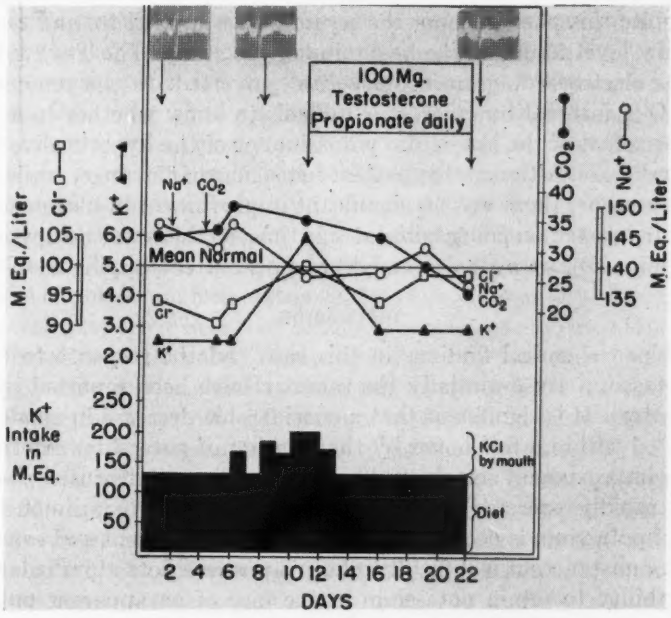


FIG. 4. The effects of oral potassium chloride and intramuscular testosterone propionate on the serum electrolytes and on the electrocardiogram.

previously noted. After seven days with no extra potassium added to the diet, the CO_2 combining power showed an increase and the serum chloride a decrease. The serum potassium remained at 2.5 milliequivalents per liter. At this time KCl in an amount varying from 3 to 5 grams per day was added to the diet. After five days the serum potassium, chloride and sodium concentrations were essentially normal but the CO_2 combining power was still elevated. The electrocardiogram on the ninth day revealed an increase in the height of the T-wave consistent with an increasing serum potassium concentration. On the eleventh day daily injections of 100 milligrams of testosterone propionate were begun in order to determine whether this hormone, by virtue of its ability to cause a retention of potassium intra-

cellularly, might correct the alkalosis even if extra potassium were not given. Unfortunately, through an error, extra potassium chloride was given by mouth for two more days. As is clear in Figure 4, however, treatment with testosterone propionate for eleven days was associated with the complete restoration to normal of the CO_2 combining power of the plasma for the first time since the patient was under observation. The serum sodium and chloride at this time were also essentially normal. It is most significant that despite this phenomenon, the serum potassium fell to, and remained at the low level found at the beginning of the study. The T-wave in lead II of the electrocardiogram again became inverted. In the presence of a normal Q-T interval however, it is difficult to know whether to interpret this as an effect of the low serum potassium or of the hypertensive cardiovascular disease. Although the patient felt somewhat stronger while receiving testosterone, there was no significant improvement in his general condition. He insisted on going home at this time, so there was no opportunity to determine how soon alkalosis might reappear after stopping testosterone.

DISCUSSION

The blood chemical findings in this case and the response to therapy with potassium are essentially the same as have been reported by other investigators. It is significant that a considerable decrease in alkalosis can be effected within a few hours by the infusion of potassium chloride, but that the improvement so achieved is transient since the infused potassium is very rapidly excreted in the urine. Apparently a continuously high intake of potassium is necessary to maintain improvement and even under these circumstances it is difficult to keep a positive potassium balance (5). This inability to retain potassium in the face of an apparent potassium deficiency contrasts with the situation in other conditions associated with hypokaliemia in which potassium is retained rather avidly (14). This is true in those cases in which there is a relatively or absolutely deficient intake of potassium with either a normal or temporarily excessive output, as in patients undergoing major surgical procedures, in patients having vomiting or diarrhea, or during the treatment of diabetic acidosis. The inability to retain potassium very long in Cushing's disease suggests that the potassium deficiency must be due to an active, continuous process, either preventing the passage of potassium into the cell or acting directly on the kidney to increase renal elimination of that ion, so that a chronic insufficiency results unless the intake is kept high. The latter interpretation would seem more likely in view of the effectiveness of a sustained increase in the potassium intake. Kepler (5) has shown that if the potassium intake is large enough, not only positive potassium but also positive nitrogen balances are achieved. Serum and muscle potassium con-

centrations are restored to normal by these measures. These results suggest that the difficulty in retaining nitrogen in Cushing's syndrome may be dependent in part on the potassium deficiency.

The striking results when testosterone was given support the interpretation that the alkalosis is secondary to an intracellular potassium deficit, since this hormone is known to produce active potassium and nitrogen retention. Unfortunately, however, until this study can be repeated with the balance technique, we have no proof that potassium retention did indeed take place. We can only point to the studies of Talbot (11) showing that testosterone will decrease the urinary excretion of potassium and at the same time may cause a sharp fall in the serum potassium in normal individuals and in a patient with Addison's disease; and the studies of Reifenstein *et al.* (15) indicating that testosterone will induce positive nitrogen and potassium balances in Cushing's syndrome. The mechanism of action of testosterone here is not established, but it seems reasonable that it might be at the level of the cell rather than the kidney, enabling retention of potassium from extracellular fluid to occur at a lower gradient. In the case reported here, the alkalosis was corrected even though the serum potassium concentration decreased to a low level.

The changes in the electrocardiogram which occurred in this case require no special comment as they are in accord with other reports on the relation between potassium and the electrocardiogram. Because of the hypertensive cardiovascular disease the presence of an inverted or isoelectric T-wave with a normal Q-T interval on two occasions, once with a low serum potassium but no alkalosis and once with a normal serum potassium, is difficult to interpret in relation to intracellular or extracellular potassium concentrations.

Some comment might be made on the fact that neither this nor any reported case of Cushing's syndrome with alkalosis, hypochloremia and hypokaliemia has exhibited either the paresis commonly associated with hypokaliemia or the tetany seen in other conditions in which a comparable degree of alkalosis exists. This may be related to the observation previously reported from this clinic (16) that hypokaliemia may mask the manifestations of tetany due to a low serum ionized calcium; and, conversely, that the low ionized calcium may compensate for the effects of a low serum potassium level. In contrast to the patients previously reported with low serum potassium and calcium levels in whom administration of potassium precipitated tetany, no such phenomenon occurs in Cushing's syndrome, since the administration of potassium corrects the alkalosis and hence increases the ionized calcium. Presumably tetany occurs after vomiting because the alkalosis may precede the potassium depletion in this situation.

SUMMARY

The occurrence of hypokaliemia, hypochloremia and alkalosis in a 35-year-old man with Cushing's syndrome due to a probable adrenal cortical carcinoma is described

Rapid intravenous infusion of 28 grams of potassium chloride over five hours returned the low serum potassium and the electrocardiographic abnormalities to normal and considerably decreased, but did not abolish, the alkalosis. The infused potassium was rapidly lost in the urine and by sixty hours after infusion, the serum electrolyte pattern was approximately the same as before treatment.

Treatment with 100 milligrams of testosterone propionate intramuscularly daily for eleven days was accompanied by the disappearance of the alkalosis and hypochloremia for the first time, although the serum potassium remained at low levels. Since testosterone is known to produce a positive potassium balance, even in Cushing's syndrome, this finding is consistent with the view that the alkalosis in this syndrome is related to a deficit in intracellular potassium.

Addendum

Since this manuscript was submitted for publication another opportunity was afforded us to correct a similar alkalosis with testosterone propionate. In a 48-year-old man with rheumatoid arthritis there developed alkalosis (CO_2 combining power 33.0 mEq./L.) and hypokaliemia (serum K^+, 2.18 mEq./L.) with a serum chloride value of 95.7 mEq./L., during therapy with ACTH. Administration of 75 mg. of testosterone propionate daily for two days and 50 mg. daily for two days resulted in a progressive fall in the CO_2 combining power to 23.0 mEq./L., the serum potassium rising to 2.92 mEq./L. and the chloride to 105.9 mEq./L. ACTH dosage and potassium intake were unchanged.

REFERENCES

1. McQuarrie, I.; Johnson, R. M., and Ziegler, M. R.: Plasma electrolyte disturbance in patient with hypercorticoadrenal syndrome contrasted with that found in Addison's disease, *Endocrinology* **21**: 762–772 (Nov.) 1937.
2. Anderson, E.; Haymaker, W., and Joseph, M.: Hormone and electrolyte studies of patients with hyperadrenocortical syndrome (Cushing's syndrome), *Endocrinology* **23**: 398–402 (Oct.) 1938.
3. Willson, D. M.; Power, M. H., and Kepler, E. J.: Alkalosis and low plasma potassium in a case of Cushing's syndrome: a metabolic study, *J. Clin. Investigation* **19**: 701–707 (Sept.) 1940.
4. Cluxton, H. E., Jr.; Bennett, W. A.; Power, M. H., and Kepler, E. J.: Cushing's syndrome without adenomatous or hyperplastic changes in the pituitary body or adrenal cortices and complicated by alkalosis: report of case with necropsy, *J. Clin. Endocrinol.* **5**: 61–69 (Feb.) 1945.

5. KEPLER, E. J.; SPRAGUE, R. G.; CLAGETT, O. T.; POWER, M. H.; MASON, H. L., and ROGERS, H. M.: Adrenal cortical tumor associated with Cushing's syndrome. Report of a case with metabolic studies and remarks on the pathogenesis of Cushing's syndrome, *J. Clin. Endocrinol.* **8:** 499–532 (July) 1948.
6. KEPLER, E. J.; SPRAGUE, R. G.; MASON, H. L., and POWER, M. H.: The pathologic physiology of adrenal cortical tumors and Cushing's syndrome, *Recent Progr. Hormone Research* **2:** 345–391, 1948.
7. KEPLER, E. J.: Cushing's disease: a primary disorder of the adrenal cortices? *Ann. New York Acad. Sci.* **50:** 657–678, 1949.
8. DARROW, D. C.: Body-fluid physiology: the relation of tissue composition to problems of water and electrolyte metabolism, *New England J. Med.* **233:** 91–97 (July 26) 1945.
9. DARROW, D. C.; SCHWARTZ, R.; IANUCCI, J. F., and COVILLE, F.: The relation of serum bicarbonate concentration to muscle composition, *J. Clin. Investigation* **27:** 198–209 (March) 1948.
10. TALBOT, N. B.; BUTLER, A. M., and MACLACHLAN, M.: Effect of testosterone and allied compounds on mineral, nitrogen and carbohydrate metabolism of girl with Addison's disease, *J. Clin. Investigation*: **22:** 583–593 (July) 1943.
11. PETERS, J. P., and VAN SLYKE, D. D.: Quantitative Clinical Chemistry. Vol. II. Methods. Baltimore, Williams and Wilkins Co., 1932, pp. 296–298.
12. BARNES, R. B.; RICHARDSON, D., and BERRY, J. W.: Flame photometry. A rapid analytical procedure, *Indust. & Engin. Chem.*, Anal. Ed. **17:** 605–611 (Oct.) 1945.
13. WHITEHORN, J. C.: A system of blood analysis. Supplement II. Simplified method for the determination of chlorides in blood or plasma, *J. Biol. Chem.* **45:** 437–448 (Feb.) 1920.
14. TARAIL, R., and ELKINTON, J. R.: Potassium deficiency and the role of the kidney in its production, *J. Clin. Investigation* **28:** 99–114 (Jan.) 1949.
15. REIFENSTEIN, E. C., JR.; ALBRIGHT, F., and WELLS, S. L.: The accumulation, interpretation, and presentation of data pertaining to metabolic balances notably those of calcium, phosphorus, and nitrogen, *J. Clin. Endocrinol.* **5:** 367–395 (Nov.) 1945.
16. ENGEL, F. L.; MARTIN, S. P., and TAYLOR, H.: On the relation of potassium to the neurological manifestations of hypocalcemic tetany, *Bull. Johns Hopkins Hosp.* **84:** 285–301 (April) 1949.

HYPERADRENALCORTICISM IN A CASE OF ACROMEGALY WITH INSULIN-RESISTANT DIABETES*

WILLIAM H. DAUGHADAY, M.D., WILLIAM E. PERRY, M.D. AND CYRIL M. MACBRYDE, M.D.

From the Department of Internal Medicine, Washington University School of Medicine and Barnes Hospital, Saint Louis, Mo.

DIABETES mellitus was recognized as a feature of acromegaly in the early descriptions of Marie (1). These original observations have been confirmed by many subsequent observers. In the 100 cases described by Cushing and Davidoff (2), glycosuria was present in 25 per cent and clinical diabetes in an additional 12 per cent. A later study of the same 100 cases and 53 additional cases of acromegaly showed the incidence of glycosuria to be 36 per cent and of clinical diabetes, 17 per cent. Thus, the occurrence of diabetes in acromegaly far exceeds that in the general population. Almost without exception the skeletal manifestations of acromegaly precede the onset of diabetes (average time interval 9.2 years) (3).

The severity of the disturbance in carbohydrate metabolism varies. In many acromegalics it is demonstrable solely by an abnormal glucose tolerance curve or by mild intermittent glycosuria. Diabetes in acromegalic patients is often mild and controlled by diet and small doses of insulin, which has suggested to some that the diabetes does not differ from pancreatic diabetes (3, 4, 5, 6, 7). However, relative ineffectiveness of insulin has been observed in many cases (2, 8, 9, 10, 11). Even genuine resistance to insulin has occurred. Ulrich (8) reported a patient with acromegaly who developed diabetes while under medical observation. Insulin was necessary for the control of the diabetes, in daily doses as high as 360 units for a short period of time. The insulin requirement rapidly decreased and finally insulin could be entirely withdrawn. Later, following an operation, diabetes reappeared and a dose of 100 units of insulin daily was required for control.

A similar acromegalic patient has been observed by Flaum and Ralli (10). This patient required a daily dose of insulin as high as 200 units to control his diabetes. The insulin requirement then decreased and eventually it was possible to omit insulin entirely for twenty days. The patient's diabetes again became more severe and insulin was required in increasing

Received for publication October 6, 1949.

* We are grateful to Dr. Charles L. Weber of Magnolia, Arkansas, the patient's private physician, who cooperated with us in these studies.

doses until a daily dose of 180 units was given. After eighteen months, insulin was again withdrawn and glycosuria remained at only 6 Gm. per day. It appears that insulin resistance may exist in the presence of relatively mild diabetes.

Another feature which is often characteristic of pituitary diabetes is illustrated by these 2 case reports, namely, a tendency for the diabetes to undergo rapid changes in severity. Onset of clinical diabetes is frequently abrupt and clinical remission may be equally sudden. As in the foregoing cases, remission may be without demonstrable cause. In other cases, however, improved carbohydrate metabolism has followed operation (2, 12), roentgen-ray therapy to the hypophysis (13), basilar meningitis (14) and vascular accidents involving the hypophysis (15).

The following case of acromegaly is reported because of the unusual severity of the diabetes and because the observed increased excretion of "cortin" suggests that there may have been a synergism of adrenal and pituitary factors operative in the cause of the diabetes.

CASE REPORT

M. K., a married truck driver, aged 37, entered Barnes Hospital in May, 1947, complaining of increasing size of his face and extremities, of four years' duration. Before the onset of his illness, the patient had been a slightly built man of aquiline features. He first noticed an enlargement of his nose and coarsening of his features at the age of 33. There had been a weight gain of 33 pounds without change in physical strength. Libido and potency were decreased. Several weeks before entry he had a severe episode of epistaxis for which he had been hospitalized in his community. His acromegalic features were recognized and he was referred to Barnes Hospital for treatment.

Prior to the onset of his acromegaly the patient had enjoyed good health. Diabetes or other known glandular diseases had not occurred in members of his family.

The patient was found to be alert and cooperative on physical examination. His facial features were coarse; his nose in particular was very large. His hands were wide and his fingers appeared short and thick (Fig. 1). Similar changes were noted in his feet. A slight dorsal kyphosis was present. The skin was thick and oily and there was a papular folliculitis which was mostly over the chest and back. Body hair was profuse and pubic and axillary hair were present in normal amounts. No defects could be demonstrated in the peripheral visual fields and the optic discs had clearly defined margins. The heart was of normal size and the lungs were clear. The blood pressure was 120 mm. of Hg systolic and 80 mm. diastolic. The muscles of the calves seemed disproportionately small in comparison to the rest of the body.

The specific gravity of the urine was 1.020 and glycosuria was not present. A trace of albumin was present and the centrifuged sediment contained many red blood cells without casts. The red blood cell count, hemoglobin concentration, white blood cell count and the differential count were within normal limits. The fasting blood sugar[1] was 120 mg. per cent. Glucose tolerance was impaired (Table 1). The basal metabolic rate was minus 10 per cent.

[1] Measured as "true blood sugar" using the Somogyi-Shaffer-Hartman method.

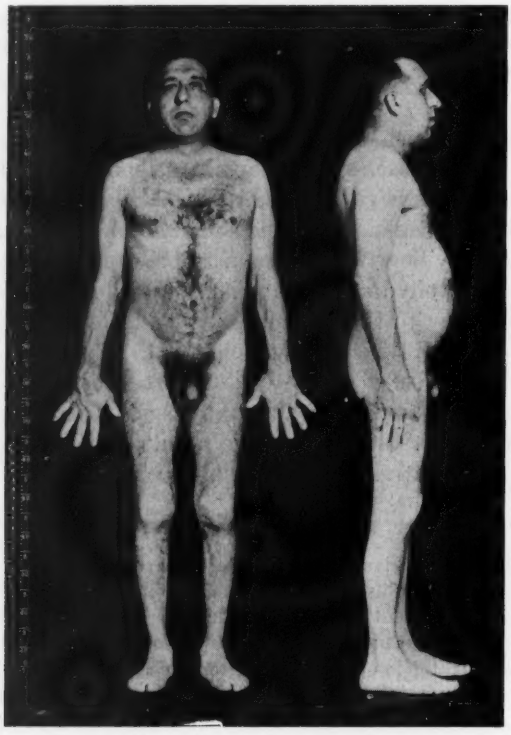


FIG. 1. L. K., showing moderate acromegalic changes. Note comparative atrophy of musculature of lower extremity.

TABLE 1. TESTS OF CARBOHYDRATE TOLERANCE AND INSULIN SENSITIVITY

Date	Substance given	Blood sugar (mg. per cent)						
		Fasting	Minutes					
			15	30	60	120	180	240
5/14/48	Glucose, 100 Gm. orally	120		165	242	240	167	
10/ 4/48	Insulin, 1/40 unit/Kg. i.v.	76	74	78	83			
3/18/49	Insulin, 1/10 unit/Kg. i.v.	146		85	101	113		
3/19/49	Insulin 14 units s.c.	180			176	166	133	119

Extensive enlargement of the sella turcica with atrophy of the posterior clinoid processes was demonstrated by x-ray examination (Fig. 2).

Radiation treatment was given to the pituitary through two lateral portals in a total dose of 4,200 roentgen units administered in fourteen treatments over a period of seventeen days. The patient returned to his home after the completion of therapy, noting a slight decrease in the soft tissues of his hands and feet. In October, 1947, "almost overnight," urinary frequency, polyuria and polydipsia developed. He lost 14 pounds in weight and in two weeks became so weakened that he was confined to bed. His physician discovered sugar in his urine and began insulin treatment. Control of his glycosuria

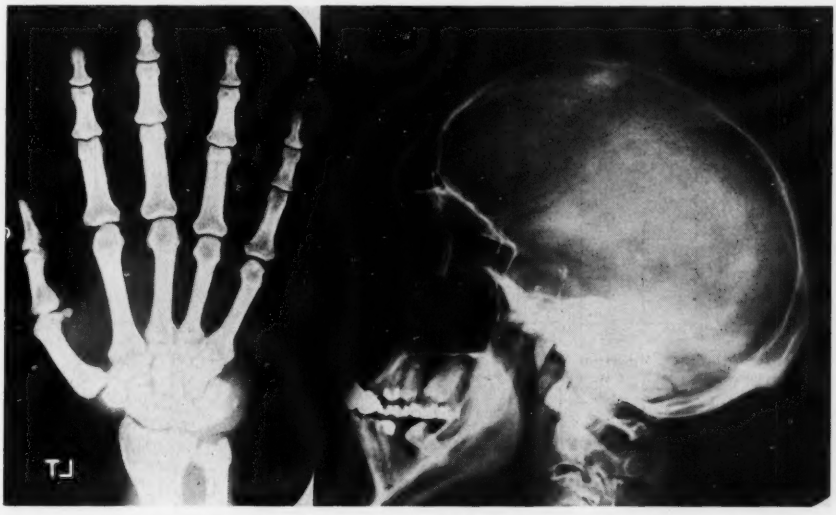


FIG. 2. X-ray films of hand and skull. Considerable soft tissue enlargement of the hand can be seen. The sella is enlarged, with atrophy of the posterior clinoid processes.

proved difficult despite increasing doses of insulin. Cramping pains in the calf muscles were troublesome and the patient returned to Barnes Hospital in December, 1947. At this time, he was receiving 20 units of protamine zinc insulin and 40 units of regular insulin daily.

On entry, the blood sugar was 358 mg. per cent and the urine contained large amounts of sugar but no acetone. Partial control of the glycosuria was achieved with 110 units of crystalline insulin and 50 units of protamine zinc insulin daily. His diet contained 126 grams of protein, 150 grams of fat and 200 grams of carbohydrate daily. Visual fields were again intact. Additional x-ray treatment was given with a total dose of 2,400 roentgen units administered in twelve treatments over a period of nineteen days. He was discharged in January, 1948 to return to his home.

Moderate glycosuria continued at home despite the relatively large insulin doses. Libido and potency which had practically disappeared at the onset of diabetes did not improve. Generalized weakness and cramping pains in his legs continued.

At the end of March, 1949, he returned to the hospital. Examination showed that

his acromegalic features had not progressed. His blood pressure was found to be 140 mm. of Hg systolic and 110 mm. diastolic. It was soon discovered that the insulin requirement for adequate control of his diabetes ranged from 300 to 650 units daily. The average daily insulin doses are presented in Figure 3. The patient was at first given separate injections of crystalline and of protamine zinc insulin. Later, the diabetes was well controlled on two injections daily of concentrated regular insulin[2] (500 units per cubic centimeter) in doses shown in Figure 3. X-ray treatment was again given to the

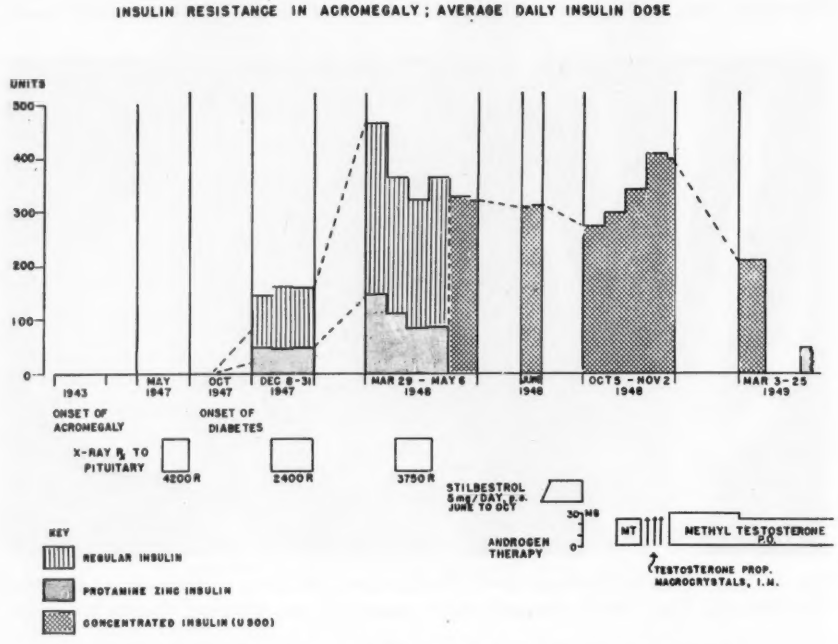


FIG. 3. Average daily dose of insulin and of other therapeutic agents.

pituitary through a vertical and an anterior portal in a total dose of 2,750 roentgen units administered in eleven treatments over a period of thirteen days.

After returning home, his glycosuria was well controlled but he had several mild episodes of sweating and nervousness before lunch.

In June, 1948, he again entered Barnes Hospital. His physical status had not changed except that his blood pressure was now found to be 160 mm. of Hg systolic and 125 mm. diastolic. Slight ankle edema was present and albumin and red cells persisted in the urine. A tachycardia not responding to digitoxin was present. Salt restriction was followed by a loss of 10 pounds of weight. At this time stilbestrol therapy was started in an attempt to inhibit pituitary function. He was given 1 mg. of diethylstilbestrol orally per day, which was gradually increased to 5 mg. daily. He continued to take 5 mg. daily from June to October, 1948. No improvement of carbohydrate metabolism was observed

[2] Kindly supplied by Dr. F. B. Peck of the Eli Lilly Company, Indianapolis, Indiana.

and he continued to require approximately the same insulin dose as before. In addition, stilbestrol aggravated his ankle edema.

Because of progressive weakness, especially in his legs, he returned to the hospital in October, 1948. Androgen therapy was started in an attempt to improve his physical strength and perhaps improve carbohydrate tolerance. At first he received methyltestosterone linguets. While in the hospital he received three injections of 25 mg. each of macrocrystalline testosterone propionate (Ciba). Upon discharge from the hospital he was instructed to take 30 mg. of methyltestosterone daily in the form of tablets.[3]

At home there was a gratifying improvement in strength and the patient's libido and potency were partially restored. There was also an improvement in his diabetes, making it possible for his physician to reduce the daily insulin dose to 240 units.

TABLE 2. DETERMINATIONS OF CHEMICAL CONSTITUENTS OF THE BLOOD AND BASAL METABOLIC RATE

	5/14/47	12/12/47	3/30/48	4/28/48	6/9/48	10/8/48	3/3/49
Nonprotein nitrogen, mg. %	18		21		23	25	28
Total protein, Gm. %		5.4	5.9		5.8	5.3	7.0
Serum albumin, Gm. %		3.5	3.1		3.7	3.7	4.0
Serum globulin, Gm. %		1.9	2.8		2.1	1.6	3.0
Serum phosphorus, mg. %				4.6	4.1	4.8	8.3
Serum calcium, mg. %				11.1			
Basal metabolic rate, %	−10	−17	−9	−4	−3	+6	+12

He re-entered Barnes Hospital at our request in March, 1949. Repeated tests revealed no glycosuria on a daily dose of 220 units of concentrated regular insulin. Several times he had episodes of hypoglycemia. On the tenth hospital day, insulin was discontinued. Thereafter only a trace of sugar was found in the urine and the fasting blood sugar levels were only moderately elevated (between 130 and 180 mg. per cent). He was discharged to his home feeling quite well on a dose of 40 units of protamine zinc insulin daily and 20 mg. of methyltestosterone tablets daily.

At no time during the long period of our observations was there any manifestation of insulin allergy or local reaction suggesting delayed insulin absorption.

Tests of carbohydrate metabolism which were performed on this patient have been summarized in Table 1. Some of the observations on the chemical constituents of the blood and the basal metabolic rate are presented in Table 2. During the period of our observation there was a slight rise in the nonprotein nitrogen level which, with the presence of abnormal urinary

[3] Kindly supplied by Roche-Organon Company, Nutley, New Jersey.

findings, probably indicates a progressive degenerative nephritis. Serum phosphorus was slightly elevated until the last admission, when the level had risen as high as 8.3 mg. per cent.

Special studies of blood lipids were performed in the laboratory of Dr. Leo Wade. During the acute phase of the diabetes, there was a lipemia and hypercholesterolemia. With improvement in the clinical status there was a return toward more normal values (Table 3).

An attempt was made to demonstrate the presence of substances in the patient's blood which inhibited the utilization of glucose. Experiments were performed by Dr. C. R. Park of the Department of Biological Chemistry, using as a test object the isolated rat diaphragm (16). The rate of utiliza-

TABLE 3. BLOOD LIPIDS

	10/29/48	3/8/49	3/21/49	Normal values
Fatty acids, mEq./liter	80.3	32.8	51.5	9–16
Lipid phosphorus, mg. %	36.4	14.6	16.4	8–11
Cholesterol, mg. %				
Total	516	257	227	160–230
Free	188	87	99	40–55
Esterified	328	170	128	120–165

tion of the glucose by the rat diaphragm in vitro was measured after the addition of the patient's serum and compared to that after the addition of serum from a normal subject. There was no demonstrable inhibition of glucose uptake by this technic.

Because of the production of diabetes in normal subjects by the administration of ACTH (17) and the vast experimental literature showing an anti-insulin effect of adrenal steroids, we attempted to evaluate adrenal function in this patient. The criteria which we used were the excretion of urinary "cortin", and 17-ketosteroids. Urinary "cortin" was measured by a method previously described (18) which depends upon the liberation of formaldehyde from a partially purified urinary extract by periodic acid oxidation. The formaldehyde liberated is distilled from the oxidation mixture and measured quantitatively, utilizing the color formed with chromotropic acid. Under normal conditions excretion of this material rarely exceeds 2 mg. a day. The excretion of 17-ketosteroids was estimated by a simplification of the method of Holtorff and Koch (19). The results obtained are presented graphically in Figure 4. The first series of assays was performed during a period of severe insulin resistance. There was a definite elevation of the urinary "cortin" and the 17-ketosteroids were at the upper limit of normal. The last studies were made after the patient had entered a period

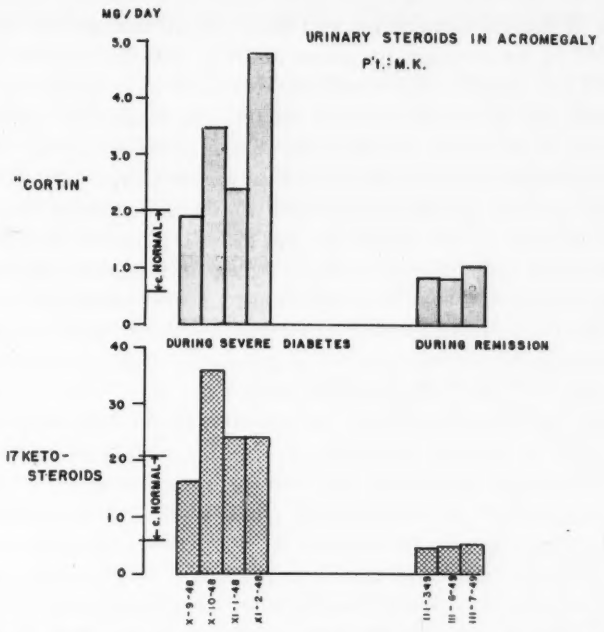


FIG. 4. Assays of urinary steroids during the period of severe diabetes with insulin resistance; and assays after the patient had entered a period of clinical remission.

of clinical remission and there was a fall of both types of compounds to normal level.

DISCUSSION

Although it has been suggested that the diabetes of acromegaly is due to a hypothalamic disturbance produced by the pressure of the pituitary tumor, this hardly seems likely. Diabetes is not a common accompaniment of other types of pituitary or hypothalamic tumors and often, as in our patient, the pituitary tumor seems entirely confined to the sella. We must therefore conclude that the diabetes is probably produced by hormonal mechanisms. Several possible ways in which the increased hormone output of the pituitary eosinophilic cells might produce diabetes have been proposed and supported with experimental or clinical evidence.

1). *The pituitary overactivity of acromegaly may interfere peripherally with glucose utilization directly or through the mediation of adrenal cortical hormones.* Insight into the possible mechanisms of production of diabetes in acromegaly has been provided by the Coris and their collaborators (16, 21, 22). They have presented evidence that pituitary substances may inhibit

the activity of muscle hexokinase and that this inhibition, like the inhibition observed in hexokinase preparation from diabetic animals, may be counteracted by insulin. These cell-free extracts have proved labile and unpredictable and others have been unable to repeat these observations (34). Park and Krahl (16), utilizing the rate of glucose uptake by the isolated rat diaphragm as a possible index of hexokinase activity, have presented evidence that the glucose-uptake inhibiting factor of the pituitary is closely associated, if not identical with, growth hormone. Stadie (35, 36) and his associates have proposed that there is an intimate combination of insulin with muscle and that this combination is prevented by the previous in vivo administration of growth hormone. In vitro inhibition of insulin action was achieved by washing rat diaphragms with crude pituitary extracts but not with purified growth hormone.

The peripheral utilization of glucose may be further depressed in acromegaly if there is increased secretion of adrenocorticotropic hormone. An inhibition of glucose utilization has been produced in glucose-fed rats by the administration of adrenal cortical extract (23). In the production of diabetes in normal human subjects by ACTH, glycosuria does not seem to be related either in time or in degree to the loss of body protein and seems to be the result of a depressed capacity to utilize glucose (17).

The possibility of adrenal hyperactivity in acromegaly was suggested by Cushing and Davidoff (24) in their comprehensive report of 4 autopsies on acromegalic patients. In all cases the adrenal glands were enlarged and in 3 cases contained adenomata. It seems likely that the eosinophilic pituitary cells are not solely concerned with the production of growth hormone and that acromegaly need not be a "monovalent" disorder.

In careful cytologic studies of the anterior pituitary of rats following exposure to cold and unilateral adrenalectomy, Finnerty and Briseno-Casterjon (25) concluded that a reduction in the circulating adrenal hormones stimulates an increase in the number of pituitary acidophils and that these cells secrete ACTH. Albright and Elrick (26) have arrived at similar conclusions following a review of the experimental and clinical evidence pertaining to pituitary cell type and hormone production.

There have been relatively few reported assays of corticoid substances in the urine of acromegalic patients. Albright and Elrick (26) presented observations on 17-ketosteroids, 11-oxysteroids and glycogenic corticoids in 6 acromegalic patients. Excretion of these substances was generally normal and occasionally increased. Four acromegalics have been studied by Venning and Browne (27). One of the 4, with actively progressing disease of recent origin, had elevated corticoid excretion. Failure to find elevated adrenal steroid excretion in all cases may be accounted for partially

by postulating that increased adrenal cortical activity occurs only in the early stage of the disease.

2). *The pituitary may act by increasing glucose production and liberation, either directly or through the adrenal cortex.* Long, Katzin and Fry (23) emphasized the importance of this action by showing that increased carbohydrate production followed the injection of adrenal cortical steroids into normal and adrenalectomized fasting rats. Adrenal cortical steroids when administered to patients with Addison's disease have been found effective in maintaining blood glucose after fasting, presumably by the promotion of increased gluconeogenesis (37). The evidence that the pituitary may control the rate of gluconeogenesis has been extensively reviewed by Soskin and Levine (28).

3). *There may be direct pituitary influence on pancreatic function.* The possibility that such an effect might exist has been demonstrated by Anderson (29). Using an ingenious perfusing apparatus for the isolated pancreas, she has demonstrated that the release of insulin can be greatly inhibited by the addition of growth hormone to the perfusion medium.

4). *Temporary diabetes may occur through any or all of the mechanisms suggested and, if present over a sufficiently long period, exhaustion of the pancreatic islets with degeneration of the beta cells will occur, resulting in permanent diabetes.* This course of events has been produced in dogs by the administration of pituitary extracts (20) and in cats simply by the maintenance of hyperglycemia (50). Thus, in acromegaly there may be a more severe, relatively insulin-resistant diabetes during the active phase of the disease, to be followed by a less severe truly pancreatic diabetes when the stage of hormone overproduction has passed.

The explanation for the improvement in our patient's diabetes cannot be given with assurance. As noted before, it is not unusual for spontaneous improvement to occur. There is nothing in the patient's course to suggest destruction of the tumor due to a vascular occlusion or infection, such as has been observed in other cases. Improvement may have resulted from the x-ray treatment because the total amount administered was large. However, remission did not occur until many months after the last series of x-ray treatments.

The importance of the androgen therapy is difficult to assess. At the time of his original hormone assays, the patient presented clinical evidence of androgen deficiency, in that libido and potency were absent and generalized weakness was present. Since the 17-ketosteroids were in the high normal range at this time, it may be conjectured that these steroids were largely of adrenal origin and were not an index of androgenic activity. It is possible that our patient's improvement may have resulted from sup-

pression of his corticoid secretion by the androgens administered. In Cushing's syndrome, androgens have been one of the few agents which have suppressed elevated corticoid excretion (31, 32). The hormone assays which we have performed give some support to this argument.

Kinsell (33) has observed the beneficial effects of testosterone propionate in lowering the serum phosphorus and arresting growth in acromegaly and gigantism. In this regard, the rise in the serum phosphorus during clinical improvement at the time of our patient's last hospital entry is difficult to explain. The degree of renal insufficiency could hardly explain this rise.

It has been suggested that large doses of insulin may, in certain cases of insulin resistance, break the resistance. A rational physiologic explanation of such a phenomenon cannot be given. It is possible that the large amounts of insulin given to this patient may have permitted some partially exhausted beta cells to recover. We are not aware of evidence to indicate that insulin will inhibit pituitary or adrenal diabetogenic hormone production.

SUMMARY

Detailed clinical, biochemical and hormonal observations on a 37-year-old male with acromegaly and insulin-resistant diabetes are presented. The maximum daily insulin dose was 650 units and the insulin requirement for over seven months was about 300 units daily. X-ray treatment to the pituitary was given with little immediate improvement. Stilbestrol therapy was also without clinical effect. Remission in the severity of the diabetes and return of the adrenal steroid excretion to normal occurred while the patient was receiving methyltestosterone. Certain theoretic implications of this case are discussed.

REFERENCES

1. Marie, P., and Souza-Leite: Essays on Acromegaly. London, New Sydenham Society, 1891.
2. Davidoff, L. M., and Cushing, H.: Studies in acromegaly. The disturbance of carbohydrate metabolism, *Arch. of Int. Med.* **39**: 751–779 (June) 1927.
3. Coggeshall, C., and Root, H. F.: Acromegaly and diabetes mellitus, *Endocrinology* **26**: 1–25 (Jan.) 1940.
4. Colwell, A. R.: The relation of the hypophysis to diabetes mellitus, *Medicine* **6**: 1–40 (Feb.) 1927.
5. Hetzel, K. S.: Glycosuria in acromegaly, *Lancet* **1**: 440–441 (Feb.) 1926.
6. Yater, W. M.: Acromegaly and diabetes: report of six cases, *Arch. Int. Med.* **41**: 883–912 (June) 1928.
7. John, H. J.: The possible relationship between acromegaly and diabetes with a report of three cases, *Arch. Int. Med.* **37**: 489–511 (April) 1926.
8. Ulrich, H.: The antagonism between insulin and pituitary extract: its demonstration in a patient with acromegaly, *Arch. Int. Med.* **41**: 875–882 (June) 1928.
9. Ulrich, H.: Insulin in acromegalic diabetes, *Arch. Int. Med.* **43**: 785–794 (June) 1929.

10. Flaum, G., and Ralli, E. P.: A case of acromegaly studied during the course of the disease and at autopsy, *Endocrinology* **26:** 229–235 (Feb.) 1940.
11. Hart, F. D., and Greene, R.: Quiescent acromegaly with insulin-resistant diabetes, *Proc. Roy. Soc. Med.* **35:** 17–18 (Nov.) 1941.
12. Ellis, A. W.: Hyperclycemia and glycosuria in acromegaly with pathological report by Prof. H. M. Turnbull, *Lancet* **1:** 1200–1203 (June) 1924.
13. Allen, R. E., and Lisser, H.: Roentgen-ray therapy of the hypophysis in a patient with acromegaly: its effect on dextrose tolerance, *Arch. Int. Med.* **42:** 703–717 (Nov.) 1928.
14. Almy, T. P., and Shorr, E.: Disappearance of diabetes mellitus associated with acromegaly following acute mastoiditis and basilar meningitis, (abstract) *J. Clin. Endocrinol.* **7:** 455 (June) 1947.
15. Bastenie, P. A.: Guérison d'un diabète acromégalique par accident vasculaire dans l'adénome hypophysaire, *Acta clin. belg.* **1:** 63–71 (Jan.) 1946.
16. Krahl, M. E., and Park, C. R.: The uptake of glucose by the isolated diaphragm of normal and hypophysectomized rats, *J. Biol. Chem.* **174:** 939–946 (July) 1948.
17. Conn, J. W.; Louis, L. H., and Wheeler, C. E.: Production of temporary diabetes mellitus in man with pituitary adrenocorticotrophic hormone; relation to uric acid metabolism, *J. Lab. & Clin. Med.* **33:** 651–661 (June) 1948.
18. Daughaday, W. H.; Jaffe, H., and Williams, R. H.: Chemical assay for "cortin": determination of formaldehyde liberated on oxidation with periodic acid, *J. Clin. Endocrinol.* **8:** 166–174 (Feb.) 1948.
19. Holtorff, A. F., and Koch, F. C.: The colorimetric estimation of 17-ketosteroids and their application to urine extracts, *J. Biol. Chem.* **135:** 377–392 (Sept.) 1940.
20. Young, F. G.: Permanent experimental diabetes produced by pituitary (anterior lobe) injections, *Lancet* **2:** 372–374 (Aug.) 1937.
21. Colowick, S. P.; Cori, G. T., and Slein, M. W.: The effect of adrenal cortex and anterior pituitary extracts and insulin on the hexokinase reaction, *J. Biol. Chem.* **168:** 583–596 (May) 1947.
22. Krahl, M. E., and Cori, C. F.: The uptake of glucose by the isolated diaphragm of normal, diabetic and adrenalectomized rats, *J. Biol. Chem.* **170:** 607–618 (Oct.) 1947.
23. Long, C. N. H.; Katzin, B., and Fry, E. G.: The adrenal cortex and carbohydrate metabolism, *Endocrinology* **26:** 309–344 (Feb.) 1940.
24. Cushing, H., and Davidoff, L. M.: The Pathological Findings in Four Autopsied Cases of Acromegaly With a Discussion of Their Significance. New York, Rockefeller Institute for Medical Research, Monograph #22, 1927.
25. Finerty, J. C., and Briseno-Castrejon, B.: Quantitative studies of cell types in the rat hypophysis following unilateral adrenalectomy, *Endocrinology* **44:** 293–300 (April) 1949.
26. Albright, F., and Eirick, H.: An attempt to classify hormonal disorders of the hypophysis, *Tr. A. Am. Physicians* **61:** 42–53, 1948.
27. Venning, E. H., and Browne, J. S. L.: Excretion of glycogenic corticoids and of 17-ketosteroids in various endocrine and other disorders, *J. Clin. Endocrinol.* **7:** 79–101 (Feb.) 1947.
28. Soskin, S., and Levine, R.: Carbohydrate Metabolism. Chicago, Illinois, Univ. Chicago Press, 1946.
29. Anderson, E., and Long, J. A.: The hormonal influences on the secretion of insulin, *Recent Progr. Hormone Research* **2:** 209–227, 1948.

30. Dohan, F. C., and Lukens, F. D.: Lesions of the pancreatic islets produced in cats by administration of glucose, *Science* **105**: 183 (Feb. 14) 1947.
31. Venning, E. H., and Browne, J. S. L.: Effect of testosterone on the excretion of glycogenic corticoids, *J. Clin. Endocrinol.* **7**: 729–740 (Nov.) 1947.
32. Bartter, F. C.; Forbes, A. P.; Jefferies, W. M.; Carroll, E. L., and Albright, F.: The mechanism of action of testosterone in the therapy of Cushing's syndrome, (abstract) *J. Clin. Endocrinol.* **9**: 663 (July) 1949.
33. Kinsell, L. W.; Michaels, G. D.; Li, C. H., and Larsen, W. E.: Studies in growth. I. Interrelationship between pituitary growth factor and growth-promoting androgens in acromegaly and gigantism, *J. Clin. Endocrinol.* **8**: 1013–1036 (Dec.) 1948.
34. Stadie, W. C., and Haugaard, N.: The hexokinase reaction in tissue extracts from normal and diabetic rats, *J. Biol. Chem.* **177**: 311–324 (Jan.) 1949.
35. Stadie, W. C.; Haugaard, N.; Marsh, J. B., and Hills, A. G.: The chemical combination of insulin with muscle (diaphragm) of normal rat, *Am. J. M. Sc.* **218**: 265–274 (Sept.) 1949.
36. Stadie, W. C.; Haugaard, N.; Hills, A. G., and Marsh, J. B.: Hormonal influences on the chemical combination of insulin with rat muscle (diaphragm), *Am. J. M. Sc.* **218**: 275–280 (Sept.) 1949.
37. MacBryde, C. M., and de la Balze, F. A.: Pork adrenal-cortex extract: effect upon carbohydrate metabolism and work capacity in Addison's disease, *J. Clin. Endocrinol.* **4**: 287–296 (July) 1944.

IMPROVEMENT OF THE RAT OVARY HYPEREMIA TEST FOR PREGNANCY, BY ADDING A PITUITARY SYNERGIST

PAUL H. FRIED M.D. AND A. E. RAKOFF, M.D.

From the Department of Obstetrics and Gynecology, and the Endocrine Division of the Hospital Laboratories, Jefferson Medical College and Hospital, Philadelphia, Pa.

THE rat ovary hyperemia reaction has been used as the basis of a rapid pregnancy test, since it is the earliest demonstrable reaction to chorionic gonadotropin (1). The accuracy of the two-hour rat ovary hyperemia pregnancy test has varied greatly, however, being 99.5, 95.0, 84.5 and 68.8 per cent respectively in the hands of four different observers (2–5).Objections have been raised particularly to the false negative reactions in individual animals and to the lack of a sharp positive end point (4, 5, 6, 7). In a recent study (8) in our laboratory, approximately 90 per cent of the false negative reactions were attributed to the failure of an adequate response by the animals, and the remainder were thought to be due to poor urine absorption or other factors. Modifications in technic improved results slightly. A further attempt to overcome the false negative reactions by enhancing the ovarian hyperemia response to chorionic gonadotropin led to our use of an anterior pituitary extract for this purpose (9). This procedure was suggested by the well known synergistic effect of certain anterior pituitary extracts in augmenting the ovarian responses of corpora hemorrhagica and lutea formation when used in conjunction with chorionic gonadotropin (10–31) and the requirement of an anterior pituitary "prohormone" by chorionic gonadotropin in order to evoke these characteristic ovarian effects (1, 10, 11, 27, 32–35).

In the present study further data are presented concerning the synergistic effect of an anterior pituitary extract, with special reference to its usefulness in improving the rat ovary hyperemia reaction as a test for pregnancy.

METHODS AND RESULTS

Gonadotropic activity of the pituitary extract (synergist):

To determine the gonadotropic activity of the pituitary synergist,[1] a total of 22.2 units of the synergist in distilled water solution was injected

Received for publication October 31, 1949.

[1] Kindly furnished by the Schering Corporation, Bloomfield, N. J. The synergist was prepared from sheep pituitaries. The unit was defined as the amount of synergist

subcutaneously in three divided daily doses into each of 8, immature female, 22-day-old, albino rats of a strain of unknown identity and weighing 36 to 40 grams each. Autopsies were performed on 4 of the rats after thirty-six hours and on 4 after 120 hours (Table 1). Increase in weight of the uterus and ovaries, ovarian hyperemia, and growth follicles occurred in the injected rats. Corpora hemorrhagica (CH) and corpora lutea (CL) were absent and the vaginal vaults were closed. Similar but less marked ovarian and uterine effects were noted with smaller total doses, namely 1.0 and 3.0 units. With doses of 66.6 units a slight further increase in weight was produced. The gonadotropic effect of the pituitary extract, therefore, appears to be chiefly of the follicle-stimulating type.

TABLE 1. THE EFFECT OF THE PITUITARY SYNERGIST (F.S.H.) ON OVARIAN AND UTERINE WEIGHTS

Time (hours)	Pituitary extract (units)	No. of rats	Av. rat wt. (Gm.)	Av. ovarian wt. (mg.)	Av. uterine wt. (mg.)
—	none	4	39.5	14.25	16.85
36	22.2	4	36.5	31.8	36.95
120	22.2	4	39.7	45.70	32.40

Augmentation of ovarian stimulation with various gonadotropins plus pituitary extract:

The ability of the pituitary synergist to augment the ovarian response to chorionic gonadotropin,[2] pregnant mare serum[3] and equine pituitary gonadotropin[4] was evaluated on the basis of the production of CH and CL (Table 2). To determine the subminimal effective dose,[5] each gonadotropin was injected subcutaneously in three daily divided doses into 6 to 10 immature female mice, each weighing 6 to 8 grams. Autopsies were performed at ninety-six hours and the ovarian reactions of CH and CL formation were noted. The pituitary synergist, in a dosage of 33.3 units, when given alone failed to elicit CH and CL formation. When added to subminimal amounts

which, in combination with 15 I.U. of chorionic gonadotropin, will cause a fivefold increase in ovarian weight over that of the uninjected controls. In terms of the international standard of chorionic gonadotropin, there was an amount of L.H. equivalent to approximately 0.25 I.U. per 22.2 units of the synergist.

[2] A.P.L. (Ayerst, McKenna & Harrison)

[3] Anteron (Schering)

[4] Equine Pituitary Gonadotrophin (Squibb)

[5] The amount that produced CH and CL in less than 50 per cent of 6 or more mice.

TABLE 2. AUGMENTATION BY THE PITUITARY EXTRACT OF THE OVARIAN RESPONSE TO GONADOTROPINS

No. of mice	Gonadotropins	Pituitary synergist (units)	No. of mice with ovarian reactions (CH and CL)
6	—	33.3	0
6	Pregnant mare serum (I.U.) 0.3	—	0
6	" " " " 0.3	33.3	6
10	Whole pituitary (R.U.) 0.15	—	0
8	" " " 0.15	33.3	7
6	Chorionic (I.U.) 3.0	—	2
6	" " 3.0	33.3	6

of each gonadotropin, however, it increased the ovarian responses from 33 or less, to 88 or more per cent.

Augmentation by the pituitary extract of the rat ovary hyperemia effect of chorionic gonadotropin:

Immature albino female rats, 26 to 32 days old, weighing 45 to 60 grams, of a strain of unknown identity were injected intraperitoneally with a single dose of chorionic gonadotropin or of the pituitary synergist, in order to

TABLE 3. RAT OVARY HYPEREMIA REACTIONS AT TWO HOURS, WITH VARYING INTRAPERITONEAL DOSES OF CHORIONIC GONADOTROPIN OR OF THE PITUITARY EXTRACT

Chorionic gonadotropin			Pituitary extract		
Dose (I.U.)	Reaction		Dose (units)	Reaction	
	Pos.	Neg.		Pos.*	Neg.
1.00	6	2			
0.60	19	6	0.15	20	8
0.55	4	9	0.14	6	7
0.05	0	11	0.11	0	14

* Weak, and frequently transient.

evaluate the minimal effective dosage[6] (M.E.D.) of each. The rats were asphyxiated with ether in two hours and autopsies were performed. A positive reaction was the presence of one or more hyperemic ovaries. Pale or pinkish ovaries were indicative of a negative reaction.

[6] The amount that produced the rat ovary hyperemia reaction in approximately 75 per cent of 5 or more rats, as defined previously (9).

The M.E.D. of chorionic gonadotropin injected by the intraperitoneal route was approximately 0.60 I.U., whereas that of the pituitary synergist was approximately 0.15 units (Table 3). The percentage of positive results could not be increased appreciably by raising the dosage of chorionic gonadotropin to 1.0 I.U. Positive reactions did not appear with less than 0.55 I.U. of chorionic gonadotropin or 0.13 unit of the pituitary extract.

As we have previously demonstrated (9), augmentation of the ovarian hyperemia response occurs after the intraperitoneal injection of combinations of chorionic gonadotropin and the anterior pituitary extract. The percentage of positive reactions (76 per cent) with the M.E.D. of chorionic gonadotropin was increased to 100 per cent by the additional use of from 0.004 to 0.04 units of the pituitary extract. Strong positive reactions were noted in all rats injected intraperitoneally with 0.30 to 0.55 I.U. of chorionic gonadotropin, if 0.22 or more units of the synergist were added. Augmentative effects were also noted at one and a quarter, and at one hour.

Synergistic effect of the pituitary extract with chorionic gonadotropin in hypophysectomized rats:

Whether the pituitary synergist acts as a "prohormone" for the ovarian hyperemia effect of chorionic gonadotropin may be questioned because of the rapidity of the hyperemia response. Positive ovarian hyperemia responses were noted ten minutes after the injection of 50 I.U. of chorionic gonadotropin or 11.1 units of the synergist. Varying doses of chorionic gonadotropin, the pituitary "synergist," or a combination of the two preparations were injected intraperitoneally into hypophysectomized[7] and into normal immature albino female rats which were 30 days old, 50 to 60 grams in weight, and of the Sprague-Dawley strain. The rats were asphyxiated in two hours with ether. At autopsy the ovarian hyperemia reaction was determined as previously.

The ovarian hyperemia reactions were positive in the normal rats but were negative in the hypophysectomized rats injected with effective ovarian hyperemia doses of chorionic gonadotropin or of the pituitary synergist (Table 4). Combinations of chorionic gonadotropin and the pituitary synergist in similar effective doses were injected into normal and hypophysectomized rats. Positive ovarian hyperemia reactions were noted in all of the normal rats. In the hypophysectomized rats, the only positive reactions were noted after the injection of a combination of 10.0 I.U. of chorionic gonadotropin and 2.2 units of the pituitary synergist. It would

[7] Histologic examinations kindly performed by Dr. J. Leathem did not reveal any residual pituitary tissue. The animals were injected on the seventh postoperative day, as suggested by Williams (36).

therefore appear that chorionic gonadotropin requires the cooperation of an anterior pituitary lobe factor, presumably F.S.H., in order to produce the ovarian hyperemia effect. An insufficiency of the latter in some immature rats may be responsible for negative reactions after the injection of urines of pregnant women or of apparently adequate doses of chorionic gonadotropin.

TABLE 4. RAT OVARY HYPEREMIA RESPONSE IN IMMATURE NORMAL AND HYPOPHYSECTOMIZED RATS TO THE INTRAPERITONEAL INJECTION OF CHORIONIC GONADOTROPIN AND/OR THE PITUITARY EXTRACT

Chorionic gonadotropin (I.U.)	Pituitary extract (units)	Normal rats		Hypophysectomized rats	
		Pos	Neg.	Pos.	Neg.
0.50	—	0	2	0	2
5.00	—	3	0	0	2
10.00	—	4	0	0	2
—	0.40	0	2	0	2
—	0.10	3	0	0	1
—	2.20	4	0	0	3
0.50	0.04	4	0	0	2
5.00	2.20	3	0	0	1
10.00	1.10	3	0	0	1
10.00	2.20	3	0	2	0

Uses of the pituitary synergist for the three-hour rapid rat ovary hyperemia pregnancy test:

The rapid rat test was performed on each of 200 urine specimens sent to the Endocrine Division of the Jefferson Hospital Laboratories from 200 ward, clinic, and private patients. In each of the 200 cases, 2 rats were injected with the test urine alone and, for comparison, 1 or 2 rats were injected with the subminimal doses of the pituitary synergist in combination with the test urine. There were no false negative reports, and in fact, only 1 rat gave a false negative reaction in the first 100 cases in which each test urine and synergist was injected into 2 rats. For this reason, only 1 rat in each of the next 100 cases was injected with the combination of the synergist and the test urine. The 123 Friedman tests were done upon request, or for corroboration of negative results with the rat tests, since in our previous evaluation (6, 7) of the rat test without the synergist, false negative results had been noted whereas false positive results were scarce. A clinical diagnosis, for corroboration, was obtained three to six months later in all cases.

Details of the technic used in the pregnancy tests:

Two 26 to 32-day-old female albino rats weighing 45 to 60 grams, of a strain of unknown identity were injected intraperitoneally with a single dose of 2.0 cc. of an unaltered urine, of which a morning specimen was preferred. For comparison, the urine in combination with 0.04 unit of the pituitary synergist was injected into 1 or 2 rats. The rats were asphyxiated in three hours[8] with ether. Autopsy was performed with particular care to avoid hemorrhage and ovarian irritation, about three minutes after the death of the animals. The ovaries were examined immediately. A result was positive in the presence of one or more hyperemic ovaries, light to dark crimson in color, without regard to ovarian enlargement or follicle changes. The hyperemic reaction was reported as being strong, moderate, or weak.

TABLE 5. COMPARISON OF THREE-HOUR RAT HYPEREMIA PREGNANCY TEST, WITH AND WITHOUT ADDITION OF PITUITARY SYNERGIST

Diagnoses	Without pituitary synergist		With pituitary synergist	
	Pos.	Neg.	Pos.	Neg.
Normal Pregnancy	66	6	71	1
Disturbed Pregnancy	22	5a	25	2*
Not Pregnant	0	101	0	101

* Two incomplete abortion cases in which reactions to the Friedman test and to the rat test with and without synergist, were negative. A very small amount of tissue was obtained by D. & E.

The use of the synergist decreased the number of false negative reactions (Table 5) and false negative and weak positive reactions. In the 99 pregnancy cases the percentage accuracy with urine alone was 91 and with the urine and pituitary extract it was 99. In the 72 cases of normal pregnancy the percentage accuracy with urine alone was 91.7 and with the addition of the synergist it was 98.6. In the 27 cases of disturbed pregnancy the accuracy with urine alone was raised from 88.9 to 100 per cent by the addition of the synergist. The one false negative result with the synergist occurred with the use of only 1 rat. Of the 198 rats injected with the pregnancy urine alone, 40 (20.2 per cent) had negative reactions, whereas of the 149 rats injected with the synergist and pregnancy urine, only 3 (2.0 per cent) had negative reactions. The addition of the pituitary synergist also increased

[8] Since the period of maximum ovarian hyperemia response occurs three to six hours after injection, the animals may be killed at four or five hours, in order to overcome a possible urine absorption factor in questionable cases. On the other hand, when rapidity is desirable, as in possible ectopic pregnancy, we have recently shown (37) that a high percentage of correct results can be obtained in one hour.

the sharpness of the positive end point. Light crimson positive reactions, which may be difficult to differentiate from the pinkish-red doubtful and reddish-pink maximum negative reactions, were noted in 22 (13.9 per cent) of the 158 positive reactions to the combination of pregnancy urine and the synergist. It is also significant that in this study the accuracy with the use of only 1 rat was 99 per cent in 100 cases after the injection of a combination of test urine and the synergist.

In the 101 nonpregnant women there were no false positive results with the rat test, whereas in the 97 rabbits injected with urine from these patients, there were 2 false positive results. There were 4 false negative reactions in the 22 rabbits injected with pregnancy urines. In 3 pregnancy cases, positive results with the rat test were noted after the injection of urine and synergist at two, two, and five days respectively after the expected date of the first missed menses, whereas results with the Friedman and with the three-hour rat tests with urine alone were negative.

SUMMARY

The chief objection to the otherwise advantageous two-hour rat ovary hyperemia test for pregnancy has been the large number of false negative results which were primarily due to "non-reactor" rats. A pituitary extract, having a gonadotropic effect chiefly of the follicle-stimulating type, was found to augment the ovarian hyperemia response to chorionic gonadotropin. It was also required in addition to chorionic gonadotropin in order to evoke the ovarian hyperemia effect in hypophysectomized rats.

In a series of 200 three-hour rat ovary hyperemia pregnancy tests, the addition of the pituitary extract to the test urine increased the accuracy to 99.5 per cent, compared with 95.5 per cent noted with the use of urine alone. In the 99 pregnancy cases the accuracy was increased from 91.0 to 99.0 per cent by the addition of the extract. The percentage of false negative reactions in rats injected with pregnancy urines alone was 20.2, whereas with the pregnancy urines and the pituitary synergist it was 2.0. Another objection to the usual rat ovary hyperemia test, namely the lack of a sharp positive end point, was overcome by the use of the pituitary extract. The incidence of a light crimson color, which is a borderline reaction, was decreased from 13.9 to 1.4 per cent by the addition of the synergist. The percentage accuracy of the rat test with pituitary synergist was also greater than that of the Friedman test.

REFERENCES

1. Zondek, B., and Sulman, F.: The mechanism of action and metabolism of gonadotropic hormones in the organism, *in* Vitamins and Hormones, New York, Academic Press, 1945, vol. 3, p. 328.

2. KUPPERMAN, H. B., and GREENBLATT, R. B.: The two hour pregnancy test, *South Med. J.* **39:** 158–165 (Feb.) 1946.
3. SALMON, U. J.: Personal communication.
4. ZONDEK, B.; SULMAN, F., and BLACK, R.: The hyperemia effect of gonadotropins on the ovary and its use in rapid pregnancy test, *J.A.M.A.* **128:** 939–944 (July 28) 1945.
5. BUNDE, C. A.: An evaluation of the pregnancy test based on ovarian hyperemia in the immature rat, *Am. J. Obst. & Gynec.* **53:** 317–320 (Feb.) 1947.
6. FRIED, P. H.: An evaluation of the two hour rat test for pregnancy, *Am. J. Obst. & Gynec.* **54:** 689–693 (Oct.) 1947.
7. FRIED, P. H.: The two hour pregnancy test based on rat ovary hyperemia, *West. J. Surg.* **56:** 552–555 (Oct.) 1948.
8. FRIED, P. H.: Factors influencing the rat ovary hyperemia reaction as a test for pregnancy, *Am. J. Obst. & Gynec.* **57:** 868–877 (May) 1949.
9. RAKOFF, A. E., and FRIED, P. H.: Enhancement of effect of chorionic gonadotrophin on ovarian hyperemia in the rat by pituitary extract, *Proc. Soc. Exper. Biol. & Med.* **66:** 491–492 (Nov.) 1947.
10. EVANS, H. M.; MEYER, K., and SIMPSON, M. E.: Relation of prolan to anterior hypophyseal hormones, *Proc. Soc. Exper. Biol. & Med.* **28:** 845–847 (May) 1931.
11. EVANS, H. M.; MEYER, K., and SIMPSON, M. E.: Relation of prolan to anterior hypophyseal hormones, *Am. J. Physiol.*, **100:** 141–156 (March) 1932.
12. EVANS, H. M.; SIMPSON, M. E., and AUSTIN, P. R.: Hypophyseal substance giving increased gonadotrophic effects when combined with prolan, *J. Exper. Med.* **57:** 897–905 (June) 1933.
13. EVANS, H. M.; SIMPSON, M. E., and AUSTIN, P. R.: Further studies on hypophyseal substance giving increased gonadotrophic effects when combined with prolan, *J. Exper. Med.* **58:** 545–559 (Nov.) 1933.
14. EVANS, H. M.; PENCHARZ, R. I., and SIMPSON, M. E.: Repair of the reproductive system of hypophysectomized female rats by combinations of an hypophyseal extract (synergist) with pregnancy-prolan, *Endocrinology* **18:** 601–606 (Sept.–Oct.) 1934.
15. EVANS, H. M.; PENCHARZ, R. I., and SIMPSON, M. E.: Maintenance and repair of reproductive system of hypophysectomized male rats by hypophyseal synergist, pregnancy-prolan and combinations thereof, *Endocrinology* **18:** 607–618 (Sept.–Oct.) 1934.
16. EVANS, H. M.; MEYERS, K., and SIMPSON, M. E.: Memoirs. Berkeley, California, University of California Press, 1933, vol. 2, (section xii), p. 397.
17. EVANS, H. M.; SIMPSON, M. E.: TOLKSDORF, S., and JENSEN, H.: Biological studies of the gonadotropic principles in sheep pituitary substance, *Endocrinology* **25:** 529–546 (Oct.) 1939.
18. LEONARD, S. L., and SMITH, P. E.: Responses of reproductive system of hypophysectomized rats to injections of pregnant urine extracts: the female, *Anat. Rec.* **58:** 175–203 (Jan. 25) 1943.
19. LEONARD, S. L.: Increased stimulation of immature rat ovaries by combined injections of prolan and hypophyseal sex hormone, *Proc. Soc. Exper. Biol. & Med.* **30:** 403–404 (Dec.) 1932.
20. LEONARD, S. L., and SMITH, P. E.: Hypophyseal-like qualities of gonadotropic principle found in urine of certain animals, *Am. J. Physiol.* **108:** 22–32 (April) 1934.
21. GUSMAN, H., and GOLDZIEHER, M. A.: Synergism between pituitary extracts and chorionic gonadotropins, *Endocrinology* **29:** 931–933 (Dec.) 1941.

22. Gusman, H., and Goldzieher, M. A.: Synergism between pituitary extracts and chorionic gonadotropins, *J. Clin. Endocrinol.* **2:** 468–470 (July) 1942.
23. Fevold, H. L.; Hisaw, F. L.; Hellbaum, A., and Hertz, R.: Anterior lobe or anterior lobe-like sex hormone combinations on growth of ovaries of immature rats, *Proc. Soc. Exper. Biol. & Med.* **30:** 914–916 (April) 1933.
24. Fevold, H. L.; Hisaw, F. L.; Hellbaum, A. A., and Hertz, R.: Sex hormones of anterior lobe hypophysis: further purification of follicular stimulating factor and physiological effects on immature rats and rabbits, *Am. J. Physiol.* **104:** 710–723 (June) 1933.
25. Fevold, H. L., and Hisaw, F. L.: Interactions of gonad stimulating hormones in ovarian development, *Am. J. Physiol.* **109:** 655–665 (Oct.) 1934.
26. Fevold, H. L.: The gonadotropic hormones. Cold Spring Harbor Symposia on Quantitative Biology, 1937, vol. 5, pp. 93–103.
27. Collip, J. B.; Selye, H.; Anderson, E. M., and Thomson, D. L.: Production of estrus: relationship between active principles of placenta and pregnancy blood and urine and those of anterior pituitary, *J.A.M.A.* **101:** 1553–1556 (Nov. 11) 1933.
28. Collip, J. B.; Selye, H., and Thomson, D. L.: The loss of sensitivity to gonadotropic hormone, *Am. J. Physiol.* (Proceedings) **109:** xxii, 1934.
29. Collip, J. B.; Selye, H.; Thomson, D. L., and Williams, J. E.: Replacement of gonadotropic action of pituitary in the hypophysectomized rat, *Proc. Soc. Exper. Biol. & Med.* **30:** 665–667 (Feb.) 1933.
30. Bischoff, F.: Influence of delayed resorption on specific gonadotropic augmentation, *Endocrinology* **28:** 611–614 (April) 1941.
31. Chamorro, A.: Sur un extrait hypophysaire, dépourvu d'hormones gonadotropes, qui donne une action synergique avec le prolan, *Comp. rend. Soc. de biol.* **130:** 1195–1197, 1939.
32. Reichert, F. L.; Pencharz, R. I.; Simpson, M. E.; Meyer, K., and Evans, H. M.: Ineffectiveness of prolan in hypophysectomized animals, *Proc. Soc. Exper. Biol. & Med.* **28:** 843–844 (May) 1931.
33. Reichert, F. L.; Pencharz, R. I.; Simpson, M. E.; Meyer, K., and Evans, H. M.: Relative ineffectiveness of prolan in hypophysectomized animals, *Am. J. Physiol.* **100:** 157–161 (March) 1932.
34. Smith, P. E., and Leonard, S. L.: Effects of injecting pregnancy urine extracts in hypophysectomized rats; female, *Proc. Soc. Exper. Biol. & Med.* **30:** 1248–1249 (June) 1933.
35. Jensen, H.; Simpson, M. E.; Tolksdorf, S., and Evans, M. E.: Chemical fractionation of gonadotropic factors present in sheep pituitary, *Endocrinology* **25:** 57–62 (July) 1939.
36. Williams, P. C.: Studies of biological action of serum gonadotrophin: decline in ovarian response after hypophysectomy, *J. Endocrinol.* **4:** 127–130 (Jan.) 1945.
37. Fried, P. H., and Rakoff, A. E.: The one hour rat ovary hyperemia pregnancy test with synergist, *J.A.M.A.* **141:** 25 (Sept. 3) 1949.

STUDIES ON THE STABILITY OF EQUINE GONADOTROPIN IN CRUDE AND IN PURIFIED FORM

H. H. COLE, PH.D., H. GOSS, PH.D. AND JAMES BODA, B.S.

From the Division of Animal Husbandry, University of California, Davis, California

IN STUDYING the biologic and chemical properties of equine gonadotropin, a factor of immediate concern is the stability of the hormone under varying conditions. Furthermore, for clinical purposes, it is desirable that the hormone be highly purified. Several reports reviewed by Østergaard (1) and Leathem (2) indicate that allergic response or antigonadotropin formation may occur following the use of certain commercial preparations. Thus, the feasibility of further purification should be explored. Earlier studies (3) have shown that the hormone can be prepared in a highly purified form without excessive loss. The present paper is concerned with the stability of purified extracts as compared to that of crude pregnant mare serum.

METHODS AND MATERIALS

These studies were initiated in 1934. For all of the assays on crude pregnant mare serum, the method of Cole, Guilbert, and Goss (4) was used. Some of the purified extracts were tested by this method and some by the more accurate method suggested by Cole and Erway (5). For assay, all powders were weighed on a semimicrobalance and diluted with saline.

The rats used in the early part of our work were animals of the Long-Evans strain produced by random breeding. In our more recent work, rats of the same strain with a coefficient of inbreeding approximating 95 per cent were used. Preliminary studies indicate that a considerable increase in precision can be obtained by the use of inbred animals.

The hormone powders were regularly stored at room temperature with maximal fluctuations ranging from 60° to 90° F. The crude pregnant mare sera and the purified extracts in solution were kept in the refrigerator at 38° to 40° F.

RESULTS

Stability of equine gonadotropin in crude pregnant mare serum

Our first studies concerned the stability of the hormone in crude pregnant mare serum treated by three different methods to prevent bacterial con-

Received for publication October 24, 1949.

tamination. The first method consisted simply of passing the serum through a Berkefeld filter; the second method was to filter and then add merthiolate to a concentration of 0.02 per cent; the third was filtering and adding phenol to make 0.1 per cent. The samples were assayed and then stored in the refrigerator for six years before retesting.

A comparison of the potencies before and after storage is shown in Table 1. In addition to the levels of injection shown in the table, 0.01 ml. of serum (before storage) was injected and none of 6 rats responded. Thus,

TABLE 1. EFFECT OF VARIOUS TREATMENTS TO PREVENT BACTERIAL CONTAMINATION, ON THE STABILITY OF EQUINE GONADOTROPIN IN CRUDE PREGNANT MARE SERUM.

Six rats were used at each dosage level with autopsy 120 hours after a single injection. The effect of the treatment was determined after a six-year interval.

Treatment	Response to the following dosages			
	0.02 ml.	0.04 ml.	0.32 ml.	1.0 ml.
	Av. no. of ripe follicles or corpora	Av. no. of ripe follicles or corpora	Av. wt. of ovaries	Av. wt. of ovaries
None (before treatment)	4.3	7.0	61.3	114.5
Filtered	6.9	8.2	53.3	86.5
Filtered, plus 0.02 per cent merthiolate	2.6	7.5	51.3	76.3
Filtered, plus 0.1 per cent phenol	2.0	6.5	30.2	48.2

since 0.02 ml. produced an average of more than 3 corpora lutea per rat, the original serum contained 50 I.U. per ml. (4). By the weight method of Cole and Saunders (6), it contained 36 I.U. per ml. Very little loss in activity occurred over a six-year period in the sample treated by filtration alone or in the sample filtered and treated with merthiolate. Although the difference in response to these stored samples as compared to the sample tested before storage is within the experimental error in assay, the results suggest a loss in potency of approximately 25 per cent. The sample treated with phenol decreased in potency about 50 per cent.

These studies indicate, therefore, that equine gonadotropin in crude pregnant mare serum is very stable if stored under refrigeration and if precautions are taken to prevent bacterial contamination. As a matter of fact, we have experienced similar results with many other sera treated by filtration.

Stability of purified gonadotropin in liquid or powdered form

All extracts studied possessed an original activity between 1,000 and 7,000 I.U. per mg. Most extracts, liquids or powders, showed some loss in activity after storage (Table 2). Tests on only 1 liquid preparation were made beyond six months of storage. This extract, 17-76-4, lost 85 per cent of its original activity in 114 months, even though it showed no loss during the first five months. As certain extracts show no loss during the first few

TABLE 2. STABILITY OF PURIFIED EQUINE GONADOTROPIC EXTRACTS, TESTING 1,000 TO 7,000 I.U. PER MG. TOTAL SOLIDS, STORED IN LIQUID OR POWDERED FORM.

Stored as liquids				*Stored as powders*			
Extract No.	Original potency in I.U. per mg.	Length of storage in months	Per cent loss in potency	Extract No.	Original potency in I.U. per mg.	Length of storage in months	Per cent loss in potency
17–75–3	7,000	6	None	15–111–1	3,000	2	33
						3	33
15–19–2	5,000	6	30			19	33
17–76–4	3,700	3	None	15–111–2	1,000	19	10
		5	None				
		43	40	18–95–2	1,100	8	40
		114	85			52	25
17–70–4	2,000	6	50	18–95–1	1,000	9	30
						26	20
						76	35

months of storage, it appears that a study of factors affecting stability would be fruitful. Some preliminary studies indicate that pH of the solution is an important factor.

Most of the assays on the extracts shown in Table 2 were performed by the minimal response method of Cole, Guilbert, and Goss (4). More extensive observations were made on 2 powders,[1] 35D and C6-6, using the 48-hour method of assay suggested by Cole and Erway (5). The results are shown in Table 3. For the first three tests on 35D, random-bred rats were used; the standard errors of the means are much greater than for the inbred animals. The variation between these initial tests is much greater, therefore,

[1] 35D is a preparation kindly supplied us by Dr. Erwin Schwenk of Schering Corporation, Bloomfield, N. J.

than for subsequent tests using inbred animals. This is unfortunate, in that we do not have a good initial test for comparison. Nevertheless, the loss in activity during the first year was apparently very slight. The tests during the second year of storage indicate a slight loss.

The other extract, C6-6, remained very constant in activity over the 87 weeks of the storage period. On two occasions, duplicate tests were run using the same dilution to provide information on the extent of animal variation.

TABLE 3. STABILITY OF TWO EQUINE GONADOTROPIC POWDERS, 35D AND C6-6, WITH ASSAY BASED ON THE AVERAGE WEIGHT OF 8 TO 10 PAIRS OF IMMATURE RAT OVARIES FORTY-EIGHT HOURS AFTER A SINGLE INJECTION.

The standard errors of the means are shown.

35D			C6-6		
Date of assay	Age of powder weeks*	I.U. per mg. solids	Date of assay	Age of powder weeks	I.U. per mg. solids
7–30–1946	2	1,080 ±200‡	5–18–1947	0†	2,980 ±160
10– 8–1946	12	1,640 ±240‡	5–25–1947	1	3,370 ±160
12– 4–1946	20	1,900 ±250‡	8–26–1947	15	3,160 ±150
2–10–1947	29	1,620 ± 70	8–26–1947	15	2,860 ±140
6–16–1947	48	1,040 ± 90	8–26–1947	15	2,860 ±200
7–22–1947	53	1,580 ± 80	1– 7–1948	34	2,960 ±300‡
8–25–1947	58	1,450 ± 50	1– 7–1948	34	2,560 ±240
8–25–1947	58	1,370 ± 70	1–12–1949	87	2,940 ±250
1– 7–1948	77	1,260 ±130‡			
10–27–1948	119	1,260 ±100			
1–12–1949	130	1,070 ± 50			

* Calculated from the date the sample was received.

† Powder tested two days after preparation.

‡ The animals used in this test were not inbred.

The evidence on purified extracts indicates, therefore, that the loss during storage for reasonable periods of time is not excessive. Two of 4 liquid extracts and 2 of 6 powders showed no detectable loss during the first few months. In other powders, the activity remained fairly constant after an initial loss.

SUMMARY

Crude pregnant mare serum treated by passing through a Berkefeld filter or by filtration and the addition of merthiolate showed a loss of approximately 25 per cent after a six-year storage period. The use of phenol as an antibiotic resulted in a loss of 50 per cent over this period.

Two of 4 purified extracts stored as liquids and testing more than 3,000 I.U. per mg. total solids, showed no loss during the first five months of storage at 38° to 40° F. Two other liquid extracts showed losses of 30 and 50 per cent, respectively, during the first six months of storage. Two of 6 extracts stored as powders showed no appreciable loss during a period of one year. There was a loss in activity of approximately 30 per cent in the other 4 powders, with most of the loss occurring during the first six months.

The loss in activity of purified extracts was not sufficiently large to preclude their use for clinical purposes.

REFERENCES

1. ØSTERGAARD, E.: Antigonadotrophic Substances. Experimental and Clinical Studies on the Formation of Antigonadotrophic Substances Under Treatment With Gonadotrophic Hormones. Copenhagen, Ejnar Munksgaard, 1942.
2. LEATHEM, J. H.: The antihormone problem in clinical endocrine therapy, *J. Clin. Endocrinol.* **4**: 500–504 (Oct.) 1944.
3. GOSS, H., and COLE, H. H.: Further studies on the purification of mare gonadotropic hormone, *Endocrinology* **26**: 244–249 (Feb.) 1940.
4. COLE, H. H.; GUILBERT, H. R., and GOSS, H.: Further considerations of the properties of the gonad-stimulating principle of mare serum, *Am. J. Physiol.* **102**: 227–240 (Oct.) 1932.
5. COLE, H. H., and ERWAY, J.: 48-Hour assay test for equine gonadotropin with results expressed in International Units, *Endocrinology* **29**: 514–519 (Oct.) 1941.
6. COLE, H. H., and SAUNDERS, F. J.: The concentration of gonad-stimulating hormone in blood serum and of oestrin in the urine throughout pregnancy in the mare, *Endocrinology* **19**: 199–208 (Apr.) 1935.

A NEW SIMPLE METHOD FOR ACCURATE MEASUREMENT OF URINARY I^{131} AFTER TRACER AND THERAPEUTIC DOSES*

A. STONE FREEDBERG, M.D., ALVIN L. URELES, M.D., MARVIN VANDILLA, B.S.† AND MARY J. MCMANUS, B.S.

From the Medical Research Laboratories, Beth Israel Hospital; and the Department of Medicine, Harvard Medical School, Boston, Massachusetts

WE HAVE recently (1) described a method for quantitative measurement of the amount of I^{131} in the thyroid gland by external counting. It was apparent that the method was not only applicable to the external counting of I^{131} in the thyroid but likewise afforded a simple, direct measurement of I^{131} content in urine, as shown by the experimental data presented here. This new method of measurement is as accurate as the standard gamma counting technique previously reported from this laboratory, is simple, and requires minimum handling and exposure of the urine sample (2)

THE METHOD OF MEASUREMENT

Four platinum cathode Geiger-Mueller tubes were connected in a parallel electrical circuit and arranged in a horizontal plane (Fig. 1). The distance of each tube from the center was 60.0 cm. We have previously shown (3) that within a "circle" of this diameter (120.0 cm.) is a smaller concentric sphere, approximately 40 cm. in diameter, in which a source of I^{131} may be moved with a change in counting rate of not more than 5 per cent, as compared to net counts per minute obtained with the I^{131} source in the center of the circle. We have termed this central space the "free area" and have shown that its diameter is proportional to the diameter of the Geiger-Mueller tube circle (Fig 2).

In the upper left of Figure 2, four tubes are diagrammatically shown in a circle of 18 cm. radius. The tubes were arranged as in Figure 1 and were connected in a parallel electrical circuit. Net counts per minute obtained with a source of I^{131} in the center, 18 cm. from each tube, were considered

Received for publication October 17, 1949.

* This work was carried out under the contract between the Office of Naval Research, Atomic Energy Commission and the President and Fellows of Harvard College.

Support was also obtained from the Patrons of Research, Beth Israel Hospital.

† From the Radioactivity Center of the Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts. Supported by a grant in aid from the National Institute of Health.

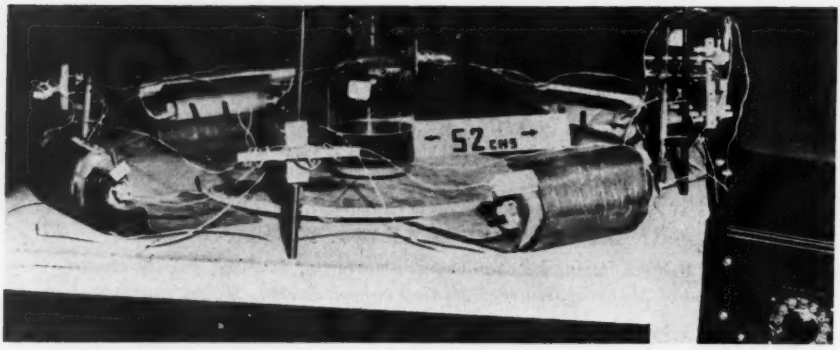


FIG. 1. Four platinum cathode Geiger-Mueller tubes connected in parallel and arranged horizontally in a circle of 60 cm. radius. A jug of urine is shown in the center of the circle.

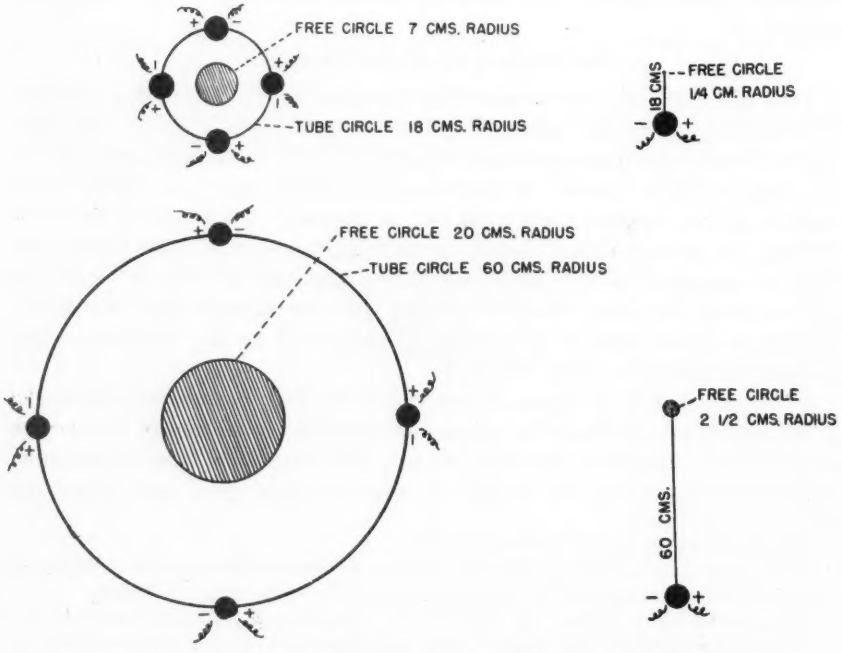


FIG. 2. The mobility of an I^{131} source in a 4-tube and a single-tube arrangement. *See* text for description.

to be 100 per cent. The source of I^{131} was moved about within the hatched central area, 7 cm. in radius, with a variation in counts of not more than 5 per cent as compared with those obtained with the source in the center. Movement of the source in the center perpendicular plane of the figure was free, 7 cm. above and below the tube level, so that the central free area approximated a sphere.

With a tube circle of 60 cm. radius (lower left, Fig. 2), a free circle 20 cm. in radius was found. In experiments with tube circles 11–60 cm. in radius, the ratio free circle/tube circle varied from 1:2.5 up to 1:3.5. In the two experiments shown in Figure 2, this ratio was 1:2.7 for the 18 cm. circle and 1:3 for the 60 cm. circle.

TABLE 1. THE EFFECT OF VOLUME (ABSORPTION, SCATTER, AND GEOMETRY) ON COUNTING RATE

Sample	I^{131} solution cc.	Diluent H^2O cc.	Net count per minute	Change in counting rate from A. per cent
A	5.0	none	4,500	
B	5.0	5.0	4,550	+1.1
C	5.0	95.0	4,500	0
D	5.0	995.0	4,250	−5.5
E	5.0	1,995.0	4,200	−6.7
F	5.0	2,995.0	4,200	−6.7

In the right of Figure 2, we have shown experiments with single tubes 18 cm. (upper right) and 60 cm. (lower right) from the source. The free circles were 0.25 cm. and 2.5 cm. in radius respectively.

The degree of freedom of a 120 cm. diameter circle to variation in volume, *i.e.*, from self-absorption and geometry effects, is shown in Table 1. Five cc. of I^{131} solution in a one-gallon glass jug was placed in the center of the circle. The net counting rate was 4,500 counts per minute. The addition of 5, 95, 995, 1,995 and 2,995 cc. of water resulted in a maximum decrease of 6.7 per cent in observed radiation. The counting rate was eight times background.

Procedure

A standard dose of 150 microcuries of I^{131}, carrier free, was administered orally to 38 patients. The urine was collected in 24-hour periods in gallon jugs containing 5 cc. of toluol and 6 to 8 pellets of sodium hydroxide.

To determine the urinary I^{131} content, the jug containing the 24-hour collection of urine was placed in the center of the circle (Fig. 1), and the time

TABLE 2. MEASUREMENT OF URINARY EXCRETION OF I^{131} BY THE 4-TUBE (PLATINUM CATHODE) AND BY THE SINGLE TUBE METHOD, FOLLOWING TRACER DOSES OF I^{131}

Exp. No.	Urine volume cc.	4-Tube method %	Beaker method %	Difference %	4-Tube method ratio of net counts/min. to background counts/min.
1	430	38.4	41.0	6.3	10
2	575	38.8	38.0	2.1	10
3	855	56.8	59.1	3.9	14
4	880	14.4	14.1	2.0	5
5	910	10.8	11.7	7.6	3
6	930	54.2	51.9	4.2	14
7	955	14.7	14.4	2.0	4
8	1,000	48.5	49.6	2.2	12
9	1,025	43.3	43.7	0.9	10
10	1,140	63.8	62.0	2.8	12
11	1,170	58.0	59.8	3.0	14
12	1,290	52.5	54.9	4.5	7
13	1,370	39.2	40.9	4.0	4
14	1,380	11.4	11.8	3.5	3
15	1,420	11.5	12.3	6.5	4
16	1,420	56.9	55.1	3.2	13
17	1,480	37.3	35.7	4.2	8
18	1,520	33.3	33.9	1.8	11
19	1,610	27.8	26.3	5.4	6
20	1,645	69.8	71.0	1.7	18
21	1,810	55.3	55.8	0.9	12
22	1,810	29.4	28.2	4.1	9
23	1,830	56.0	56.3	0.5	11
24	2,150	66.8	62.9	5.8	12
25	3,050	9.9	9.5	4.1	3
26	3,730	14.4	14.4	0	6

for 4,096 counts or multiples thereof determined. The standard of reference for each urine was a 5 to 10 cc. aliquot of the I^{131} stock solution (from which the patient's dose is taken) diluted to approximately 1,500 cc. in a gallon jug. The per cent excretion was calculated by the formula:

$$\frac{100 \times \text{net counts/min. urine}}{\frac{\text{cc. } I^{131} \text{ administered}}{\text{cc. } I^{131} \text{ in standard}} \times \text{net counts/min. of standard}}.$$

The I^{131} content of the urine sample was then determined by the Marinelli beaker method previously described (2).

RESULTS

The results are shown in Table 2 and Figures 3 and 4. The urinary I^{131} content determined by the 4-tube method varied from 9.9 to 69.8 per cent. The average difference between this method and the beaker method was 3.4 per cent, with a standard deviation of 1.8 per cent. The mean difference between the two methods was 3.2 per cent. In the observations listed in Table 2 the ratio of net counts per minute to background counts per minute varied from 3 to 18. When the counting rate was less than two times background, the per cent difference between the two methods increased to ap-

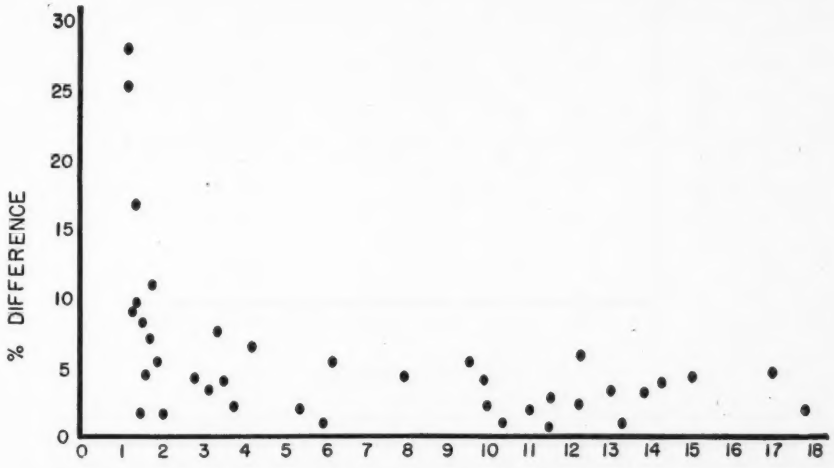


FIG. 3. The relationship of the ratio of counting rate to background to the per cent difference in urinary I^{131} excretion, as measured by the 4-tube method and by the Marinelli beaker method.

proximately 28 per cent (Fig. 3). The urine volume in these experiments varied from 430 cc. to 3,730 cc. (Table 2). It is evident (Table 2, Fig. 4) that no relationship existed between urine volume and the per cent difference in urinary I^{131} content as measured by the two methods.

COMMENT

In many clinics (4–11) urinary I^{131} excretion is utilized to estimate thyroid function and retention of this isotope. The comparative accuracy, the advantages, and the limitations of two methods employed in this laboratory for the past two years have been delineated (2). To serve as a basis of comparison for the 4-tube method described in this paper, the gamma method described by Marinelli (12) was used. This involves measuring the

gamma radiation from an aliquot of the urine sample and requires, therefore, measurement of the total urinary volume. All previously described methods have a similar requirement. It is clear from the data presented that the urinary I^{131} excretion following tracer doses can be measured by the 4-tube method with an accuracy approximating that of the beaker technique (2). The measurement of I^{131} activity by the 4-tube method, within the limits described, is independent of volume of urine, *i.e.*, absorption, scatter and geometry, and is experimental confirmation of the theoretic considerations discussed in a previous paper (3).

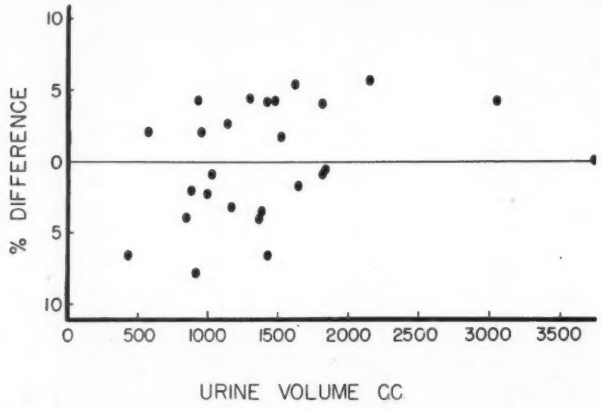


FIG. 4. The relationship between urine volume and the per cent difference in I^{131} urinary excretion as measured by the 4-tube method and by the Marinelli beaker method. Above the zero line the urinary I^{131} excretion as measured by the 4-tube method was larger than that determined by the Marinelli beaker method; below the zero line it was smaller.

As described here, the 4-tube platinum cathode method can be utilized to measure 10 or more microcuries of I^{131} in a urine volume up to 3,500 cc. The measurement of 10 or more microcuries of I^{131} in feces, or tissue other than thyroid has also been carried out by this method; the entire specimen in a suitable container (paper box) is placed in the center of the circle. Considerable increase in sensitivity is made possible by decreasing the size of the circle or increasing the number of tubes. In measuring urinary excretion of I^{131} following tracer doses in man, the 120 cm. diameter circle is useful; the central free area is sufficiently large so that independence over a wide range of urine volume is obtained (Table 1) and adequate sensitivity is retained to measure urinary excretion during the first twenty-four hours after a tracer dose of 100–150 microcuries in all patients, and during the second and third days individually in many euthyroid and

myxedematous patients. In clinics where total three-day excretion is measured, the urine can be collected in one to three bottles and measured as described.

Werner, Quimby and Schmidt (13) have pointed out the radiation hazard of radioactive iodine excreted in urine. This is most important in urine

TABLE 3. MEASUREMENT OF URINARY EXCRETION OF I^{131} BY THE 4-TUBE (COPPER CATHODE) AND BY THE SINGLE TUBE METHOD, FOLLOWING THERAPEUTIC DOSES OF I^{131}

Exp. No.	4-Tube method, net counts/min.	4-Tube method %	Beaker method %	Difference %
1	304	0.32	0.32	0.0
2	386	0.41	0.41	0.0
3	433	0.37	0.35	5.4
4	1,362	1.22	1.25	2.4
5	1,919	8.3	8.3	0.0
6	2,082	1.86	1.80	3.3
7	5,165	11.4	11.0	3.5
8	9,145	5.0	5.3	5.6
9	9,255	26.5	27.1	2.2
10	9,432	8.4	8.34	0.8
11	9,435	3.5	3.6	2.8
12	12,178	6.0	6.0	0.0
13	13,698	33.2	33.4	0.5
14	15,410	21.2	21.0	0.9
15	25,370	18.1	18.3	1.1
16	27,630	24.6	24.2	1.7
17	28,205	10.4	10.8	3.7
18	36,475	20.0	20.4	1.5
19	63,816	38.0	38.2	0.5
20	70,795	33.0	34.4	4.1
21	113,000	68.0	68.0	0.0
22	127,600	59.6	57.7	3.2
23	132,580	55.4	56.4	1.7
24	138,000	78.7	77.6	1.4
25	170,300	73.0	76.0	1.4

samples obtained from patients given larger therapeutic doses. They state, "If it is desired to determine the total volume (urine) and measure a small part, it is usually possible to obtain the patient's own assistance in handling the material during the measurement." Since the platinum cathode tubes are not commercially available, it seemed desirable to carry out experiments with other tubes. A copper screen cathode tube[1] has proved to be satisfactory for measurement of urinary I^{131} excretion following therapeutic

[1] GG 305, Sylvania Co.

doses. Four tubes, placed vertically, were connected in a parallel electrical circuit in a circle of 120 cm. diameter. The urinary I^{131} excretion was determined in the following manner: The urine sample was diluted to approximately 3,000 cc. and the radiation determined. When necessary, observed counts were corrected for resolving time losses. With this tube set-up, losses were observed with corrected rates above 40,000 per minute. The I^{131} standard was an aliquot of the dose administered to the patient and diluted in a gallon jug to 3,000 cc. The characteristics of Geiger-Mueller gamma tubes with copper cathodes are such that response or efficiency (counts per minute) is directly proportional to the quantum energy of the gamma ray emitted (14). Therefore, it becomes necessary to have essentially the same conditions of geometry and self-absorption in the standard and unknown. In the 25 experiments listed in Table 3, the final urinary volume was from 2,500 to 3,225 cc. It would appear, therefore, that dilution to approximately the same volume as the standard would be sufficiently accurate. The 3,000 cc. volume was chosen because few 24-hour urine excretions would be larger.

The I^{131} content of each urine sample was then determined by the Marinelli beaker method. In 25 consecutive comparisons (Table 3), after the oral administration of from 2.99 to 35.2 millicuries of I^{131}, carrier free, the average difference between the two methods was 2 per cent.

This method of measuring I^{131} excretion following therapeutic doses has significant advantages over methods in common use. The urine sample requires a minimum of handling and, therefore, possible exposure to radiation is limited. Since pipetting is not required and the urine volume need not be determined, glassware is limited to the gallon jugs used to collect the urine. The I^{131} in the jug may be discarded after decay has rendered it harmless.

The ratio of sensitivity of the 4-tube circle of platinum screen cathode Geiger-Mueller tubes to the 4-tube circle of copper screen cathode Geiger-Mueller tubes, each circle 120 cm. in diameter, is approximately 4 to 1. With this circle, therefore, the 4-tube copper screen cathode method can be utilized to measure accurately 40 or more microcuries of I^{131} (15). A threefold increase in sensitivity of the 4-tube copper screen cathode method has been achieved by decreasing the diameter of the copper tube circle to 90 cm. (16).

SUMMARY AND CONCLUSIONS

1. A new method for the measurement of urinary I^{131} is described. Four platinum cathode Geiger-Mueller tubes are connected in a parallel electrical circuit and arranged in a horizontal plane about the source. In the experiments described, a circle diameter of 120 cm. was used. The urinary

I^{131} content is measured by determining the radiation from the entire 24-hour collection of urine placed in the center of this circle. The standard of reference is a 5 to 10 cc. aliquot of the I^{131} stock solution from which the patient's dose is taken, diluted to approximately 1,500 cc. in a gallon jug.

2. The per cent urinary excretion was determined by this method in 38 subjects and compared to a gamma counting method (Marinelli beaker) requiring dilution and measurement of urinary volume. The average difference between the two methods was 3.4 per cent, standard deviation 1.8. The urine volumes varied from 430 to 3,730 cc. No relationship was found between urine volume and the per cent difference in urinary I^{131} content.

3. The results obtained provide experimental confirmation of the theoretic considerations which indicated the virtual freedom from absorption and geometry of the platinum cathode Geiger-Mueller tube arranged in a circle and connected in a parallel electrical circuit.

4. The adaptation of this method, utilizing copper screen cathode Geiger-Mueller tubes, for measurement of urinary excretion following therapeutic doses is described.

5. The advantages and limitations have been indicated. The minimum handling of the urine sample and limited exposure to radiation have been noted.

Acknowledgment

The authors wish to thank Dr. Robley D. Evans for his cooperation and help.

REFERENCES

1. Freedberg, A. S.; Ureles, A. L., and VanDilla, M.: Quantitative measurement, by external counting, of the I^{131} content of the thyroid gland in man, (abstract) *Fed. Proc.* **8:** 1 (March) 1949.
2. Freedberg, A. S.; Buka, R., and McManus, M. J.: Comparative value and accuracy of measurements of urinary I^{131} by beta and by gamma ray counting, *J. Clin. Endocrinol.* **9:** 841 (Sept.) 1949.
3. Freedberg, A. S.; Ureles, A., and VanDilla, M.: Studies with a new method for quantitative measurement of I^{131} content of the thyroid gland in man, (abstract) *J. Clin. Investigation* **28:** 782 (July) 1949.
4. Hertz, S.; Roberts, A., and Salter, W. T.: Radioactive iodine as an indicator in thyroid physiology. IV. The metabolism of iodine in Graves' disease, *J. Clin. Investigation* **21:** 25, 1942.
5. Keating, F. R., Jr.; Power, M. H.; Berkson, J., and Haines, S. F.: The urinary excretion of radioiodine in various thyroid states, *J. Clin. Investigation* **26:** 1138, 1947.
6. Keating, F. R., Jr.: Radioactive iodine in the study and treatment of thyroid diseases, *Postgrad. Med.* **3:** 410, 1948.
7. Haines, S. F.; Keating, F. R., Jr.; Power, M. H.; Williams, M. M. D., and Kelsey, M. P.: Use of radioactive iodine in the treatment of exophthalmic goiter, *J. Clin. Endocrinol.* **8:** 813 (Oct.) 1948.

8. OSHRY, E., and SCHMIDT, C.: Uptake and excretion measurements and their significance. Brookhaven Conference Report, July 1948.
9. CHAPMAN, E. M., and EVANS, R. D.: Treatment of hyperthyroidism with radioactive iodine, *J.A.M.A.* **131:** 86 (May 11) 1946.
10. SOLEY, M. H.; MILLER, E., and FOREMAN, N.: Graves' disease: treatment with radioactive iodine, *J. Clin. Endocrinol.* **9:** 29 (Jan.) 1949.
11. MCARTHUR, J. W.; RAWSON, R. W.; FLUHARTY, R. G., and MEANS, J. H.: The urinary excretion of radioactive iodine as an aid in the diagnosis of hyperthyroidism, *Ann. Int. Med.* **29:** 229 (Aug.) 1948.
12. MARINELLI, L. D., and HILL, R. F.: Studies on dosage in cancer therapy. Brookhaven Conference Report, July 1948.
13. WERNER, S. C.; QUIMBY, E. H., and SCHMIDT, C.: The clinical use of radioactive iodine, *Bull. New York Acad. Med.* **24:** 549, 1948.
14. BRADT, H.; GUGELOT, P. C.; HUBER, O.; MEDICUS, H.; PREISWERK, P., and SCHERRER, P.: Sensitivity of counter tubes with lead, brass and aluminum cathodes for gamma rays in energy range 0.1 to 3 MEV, *Helvet. physica acta* **19** (No. 2): 77–99, 1946.
15. FREEDBERG, A. S., and CHAMOVITZ, D. L.: Unpublished data.
16. FREEDBERG, A. S.; CHAMOVITZ, D. L.; URELES, A. L., and VANDILLA, M.: Quantitative measurement by external counting of I^{131} content of the thyroid gland in man. II. Comparison of I^{131} uptake in thyroid gland as measured by platinum screen cathode and copper screen cathode Geiger-Mueller tubes. (In preparation.)

TREATMENT OF ESTROGEN DEFICIENCY WITH ESTRADIOL PELLETS*

WILLIAM H. PERLOFF, M.D.

From the Endocrine Clinic of the Philadelphia General Hospital and the Endocrine Division of the Medical Clinics of the Hospital of the University of Pennsylvania, Philadelphia, Pa.

THE subcutaneous implantation of pure estradiol is a highly efficient method of producing estrogenic effects, both experimentally (1–7) and in the clinical management of menopausal and hypogonadal patients (8–13). In previous studies, estradiol or its esters and various other estrogens have been implanted in the form of crystals, tablets or pellets. The present report deals with a somewhat larger series of patients than hitherto described, in which clinical benefit has been marked and the duration of estrogenic effect considerably greater than anticipated from earlier studies. The prevailing concept of pituitary ovarian relationships is exemplified in a general way, but the findings include discrepancies of theoretic importance. The allowable dosage range is redetermined and the side actions to be expected are described.

MATERIAL

One hundred menopausal or hypogonadal women were treated by the subcutaneous implantation of 25-milligram pellets of pure estradiol, using either 1 or 2 pellets at each implantation, in 1 to 3 successive implantations. Most of the patients were panhysterectomized women but several showed peculiarly interesting features:

Menopausal women		
Post-hysterectomy (total)	83	
with cervical remnant		1
with oophorectomy		1
with previous endometriosis		1
Spontaneous menopause	7	
with uterine fibroid		1
Total	90	
Hypogonadal women		
secondary amenorrhea		2
primary ovarian hypogonadism		6
Turner's syndrome		2
Total	10	

Received for publication October 10, 1949.

* The author wishes to express his appreciation to Dr. Edward Henderson and Dr. William Stoner of Schering Corporation for the generous supply of estradiol pellets (Progynon) and progesterone (Proluton) used in this study and to Dr. Marvin Weinberg for his help in preparing, and to Dr. Edward Rose for reviewing this manuscript.

METHODS

The presence or absence of an intact uterus and the primary diagnosis, with its implication of hormone requirement, determined whether 1 pellet or 2 pellets would be used for the initial implantation. Implantation was made through a small skin incision in either posterior axillary line, introducing the pellets within an injector, which was than withdrawn from about them. The incision was closed with adhesive tape. The trocar may not be used to thrust the pellets through the injector, but merely serves to detain them in situ while the cannula is being withdrawn.

Evaluation of results rested wholly on clinical observation, although in many cases determinations of vaginal cornification, urinary estrogens, gonadotropins and 17-ketosteroids were carried out initially and serially. Patients were re-examined and clinical changes appraised at intervals of three to four weeks. The occurrence of one menopausal flush in a week, especially under emotional stress, was not interpreted as a return of symptoms; but one or two flushes per day, or even one daily for several days, was so counted. Certain patients were selected for reimplantation, usually at the expiration of benefit from the previous implant, or when it was evident that the response was inadequate. In 2 patients a third implantation was done.

RESULTS

Results were considered successful or unsuccessful, depending upon whether or not symptoms were completely relieved. Table 1 further distinguishes patients improved but incompletely relieved from those rare cases in which no benefit was obtained. The duration of benefit following an implantation can be gauged only for patients whose relief of symptoms was

TABLE 1. SUMMARY OF RESULTS

A. PATIENTS WITHOUT UTERINE TISSUE (PANHYSTERECTOMY)

1. *Following 1 implantation*

	1 Pellet			2 Pellets		
	Completely relieved	Incompletely relieved	Total failure	Completely relieved	Incompletely relieved	Total failure
No. of patients	52, or 91%	4, or 7%	1, or 2%	7*, or 88%	0	1 or 12%
Av. age	45.1 yrs.	44.3 yrs.	47 yrs.	43.3 yrs.		
Av. duration of relief	7.4 mos.			8.4 mos.		
Breast complaints	8, or 15%	1, or 25%	0	4, or 100%		

TABLE 1—*continued*

2. *Following 2 implantations*

	(1 and 1) pellets			(1 and 2) pellets		
No. of patients	8, or 89%	1, or 11%	0	9, or 100%	0	0
Av. age	44.0 yrs.	40.0 yrs.		44.6 yrs.		
Av. duration of relief	6.3 mos. *first* 6.0 mos. *second*			6.5 mos. *first* 8.9 mos. *second*		
Breast complaints	2, or 28% with 1st pellet 1, or 14% with 2nd pellet			2, or 22% with 1 pellet 6, or 67% with 2 pellets		

B. PATIENTS WITH UTERINE TISSUE (NOT HYPOGONADAL)

	1 pellet	(1 and 1) pellets		(1 and 2) pellets	
	Completely relieved	Completely relieved	Incompletely relieved	Completely relieved	Incompletely relieved
No. of patients	3, or 100%	2**, or 67%	1, or 33%	0	1, or 100%
Av. age	46 yrs.	27 and 43 yrs.			46 yrs.
Av. duration of relief	7.3 mos.	6 mos. *first* 6 mos. *second*			7 mos.
Breast complaints	1, or 33%	0			1, or 100%
Bleeding	2, or 67%	1**, or 50%	1, or 100%		1, or 100%

Total, 7 patients: 4 bled with 1 pellet only (incl. patient with fibroid)** and 1 bled with 2 pellets (100%) illustrating that even 1 pellet of 25 mg. of estradiol is liable to induce excessive bleeding (60% of cases).

C. PATIENTS WITH HYPOGONADISM

	1 pellet	2 pellets	(1 and 2) pellets
	Effective	Effective	Effective
No. of patients	1, or 100%	8, or 100%	1, or 100%
Av. age	33 yrs.	22.4 yrs.	25 yrs.
Av. duration of relief	11 mos.	..	3 mos. 11 mos.
Breast complaints	0	0	0
Bleeding†	0	4, or 50%	0

* Includes 1 patient with intact ovaries.

** Includes 1 patient with a uterine fibroid, in whom hysterectomy was performed during the life of the first of 2 implantations.

† Bleeding refers to objectionable side-action, not to deliberately induced "menstruation."

complete, as previously defined. Unfortunately, this cannot be determined for the considerable number of patients who are entirely well at the present time, so that an attempt to form an "average duration" suffers from the weighting of shorter durations, and yields a figure which is pessimistic compared with actual experience.

As the data will show, the symptoms of the menopause are not dependably related to urinary estrogen, gonadotropin, or 17-ketosteroid titers, nor even, over wide limits, to cornification of the vaginal smear. A few patients showed a decline of their previously high urinary gonadotropin levels with clinical relief; but, this was by no means a constant finding. Full vaginal cornification usually occurred during the first months of an implant, when symptomatic relief was complete, but there was no uniform clinical trend in patients having intermediate grades of vaginal estrus.

Young oophorectomized women generally suffered more severe menopausal syndromes than older women, and required a somewhat larger dosage of estrogen. Apart from this, the severity of symptoms does not dictate the dosage to be used, nor does a higher dosage necessarily imply a better result, as long as the minimum is as great a quantity as 25 mg. of estradiol. No difference in symptomatology or hormone requirement was noted between white and colored women, or between middle-aged and older menopausal women.

Of 100 patients, 84 received a first implantation of 1 pellet, and 16 of 2 pellets, with 8 and 1 failures, respectively. In 23 patients a second implantation was done (including 3 insufficiently benefited by the first implantation), which was successful in 20. Only 2 patients received little benefit. The duration of relief of symptoms, among those patients who had come to the end of the useful life of their implants, was 7.2 months (average) after a single pellet and 8.4 months (average) after 2 pellets. The following results were obtained in 129 separate implantations of either 1 or 2 pellets:

	Implants	*Complete relief*	*Incomplete relief*	*Total failure*
One pellet	96	89 (93%)	6 (6%)	1 (1%)
Two pellets	33	31 (94%)	0	2 (6%)

The 2 failures with 2 pellets occurred in one patient, a woman who previously had failed to respond to large doses of oral estrogens. Of practical significance is the finding that in the menopausal women studied, a single pellet was clinically effective in 89 out of 96 implantations, or in 93 per cent. It is evident that 25 mg. of estradiol suffices for the control of symptoms in a majority of menopausal women; and, as will be shown, even this dosage may give rise to difficulty if the uterus is intact.

Side actions. These consisted of breast discomfort and uterine bleeding. Mammary pain or distress occurred during the first ten to fourteen days

(occasionally up to a month) after implantation. It was evanescent in character and never sufficiently severe to influence the planning of treatment. It was absent in hypogonadal subjects. Excluding the latter patients, it occurred 15 times in 94 implantations of 1 pellet (16 per cent) and 14 times in 24 implantations of 2 pellets (58 per cent).

Uterine bleeding was a potential problem in every patient with an intact uterus, and, indeed, even in 1 patient with a cervical remnant. Hypogonad-

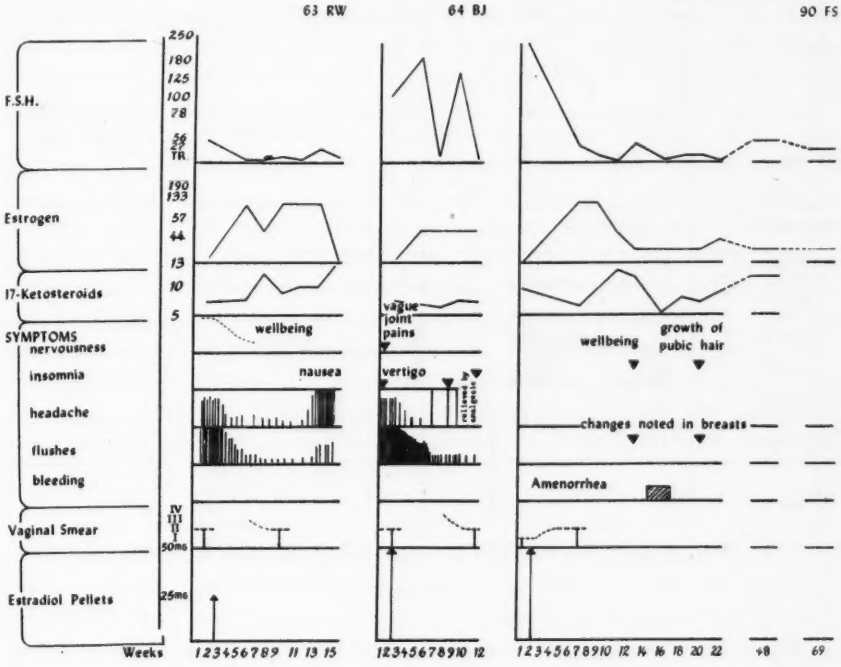


FIGURE 1

al subjects bled irregularly and in 4 of the 10 cases, incontinently. One patient with a uterine fibroid showed marked growth of the tumor and had considerable metrorrhagia until, in the fifth month of the implantation, a hysterectomy was required. Of the remaining 6 patients with a uterus (or cervix) present, 4 had undesirable bleeding at some time, and 3 of these 4 bled as a result of implantation of only a single pellet. It is evident that excessive bleeding must be anticipated in a majority of patients with the uterus intact, even if only 25 mg. of estradiol is implanted.

To control such bleeding, it was found that 25 to 50 mg. of progesterone

injected twice in three days caused bleeding to cease almost immediately; three to five days later, bleeding occurred, lasting from three to four days, following which there was no further difficulty for at least three months. In no case has it been necessary to remove a pellet or perform a curettage.

Special features. Patients treated with oral estrogens prior to implantation usually preferred the implants.

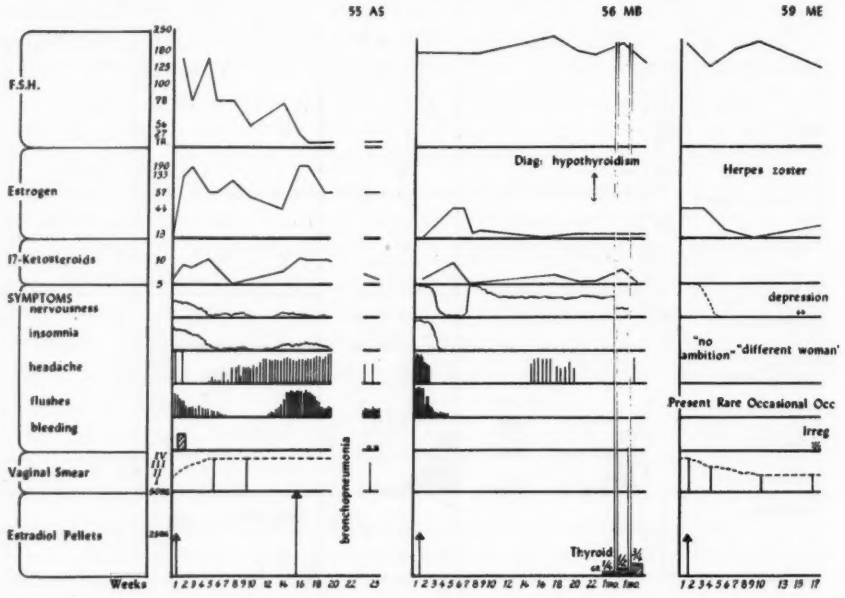


FIGURE 2

When symptoms began to recur during the first few months after implantation, they were relieved in most instances by the application of a hot water bottle to the pellet site.

The patient who had been refractory to oral estrogen, and who enjoyed little or no relief after two implantations of 2 pellets each, still experienced the same kind and intensity of mammary discomfort that occurred in responsive patients. Following each implantation, she had a urinary gonadotropic titer above 125 mouse units, while urinary estrogens were 44 and 133 mouse units respectively, suggesting adequate absorption of estrogen. In view of the mammary effects it does not appear that she destroyed the hormone with unusual facility; instead, it is likely that her estrogenic requirement was much larger than is usual in the menopausal syndrome.

The anticipated relation between urinary gonadotropins and sympto-

matic relief was better illustrated by such patients as B. J. (Case 64—Fig. 1), who showed a disappearing titer with improving condition—although there were 122 m. u. without corresponding aggravation of symptoms at a later time in this subject. More often little correspondence was apparent. In patient M. B. (Case 56—Fig. 2) gonadotropins remained uniformly high, before, during and after complete relief of severe symptoms. Patient M. E. (Case 59—Fig. 2) had very high titers, despite continuing clinical benefit from implantation of estradiol, and another patient was asymptomatic while urinary gonadotropins were as high as 364 m. u. Patient A. S. (Case 55—Fig. 2) on the other hand, had symptoms although urinary estrogens were elevated and gonadotropins reduced. Patient R. W. (Case 63—Fig. 1), never showed elevated gonadotropins.

All the hypogonadal subjects initially had no urinary estrogen, with urinary gonadotropins over 250 m. u. Menopausal symptoms developed in some for the first time when the implanted estradiol was exhausted, or when progesterone was given to induce bleeding. Bleeding could be induced in these patients as long as one year after implantation by the use of progesterone, and even spontaneous bleeding was observed in a case of Turner's syndrome and in a case of ovarian agenesis for this period of time. Estrogenic effect was apparent in patient F. S. (Case 90—Fig. 1) up to sixty-nine weeks after implantation. Computation of the rate and amount of estradiol absorption makes it appear unlikely that more than extremely small amounts of hormone remained in situ for so long a time. The explanation of this phenomenon is not apparent.

SUMMARY

One hundred women with estrogen deficiency (menopausal syndrome, 90; ovarian hypogonadism, 10) were treated by subcutaneous implantation of either 1 or 2 pellets of 25 mg. of estradiol one to three times, with a total of 129 implantations. Symptoms were relieved in 94 patients (94 per cent) for periods ranging from 4.5 to 13 months, or an average of 7.2 months with a single pellet and 8.4 months with two pellets. Considerable estrogenic activity persisted in excess of 12 months in some patients. Side actions included temporary breast discomfort, and excessive uterine bleeding in a large proportion of patients having the uterus intact. No meaningful relationship could be observed between clinical condition and urinary gonadotopin titer. Implantation is an especially suitable method for the treatment of estrogen deficiency in hysterectomized women and is preferred by most of them to oral therapy.

REFERENCES

1. Deanesly, R., and Parkes, A. S.: Influence of method of administration on effectiveness of gonadal hormones, *Chem. and Ind.* **56**: 447, 1937.

2. Deanesly, R., and Parkes, A. S.: Factors influencing effectiveness of administered hormones, *Proc. Roy. Soc., London*, s.B. **124:** 279–298 (Dec. 7) 1937.
3. Deanesly, R., and Parkes, A. S.: Further experiments on administration of hormones by subcutaneous implantation of tablets, *Lancet* **2:** 606–608 (Sept. 10) 1938.
4. Deanesly, R.: Depression of hypophyseal activity by implantation of tablets of oestrone and oestradiol, *J. Endocrinol.* **1:** 36–48 (June) 1939.
5. Emmens, C. W.: Rate of absorption of androgens and estrogens in free and esterified form from subcutaneously implanted tablets, *Endocrinology* **28:** 633–642 (April) 1941.
6. Lipschütz, A., and Vargas, L.: Absorption of estradiol and its esters from subcutaneously implanted tablets in guinea pig, *Proc. Soc. Exper. Biol. & Med.* **48:** 464–467 (Nov.) 1941.
7. Walter, R. I.; Geist, S. H., and Salmon, U. J.: Absorption rates and biologic effects of pellets of α estradiol and α estradiol benzoate in women, *Proc. Soc. Exper. Biol. & Med.* **44:** 314–318 (June) 1940.
8. Loeser, A. A.: Subcutaneous implantation of female and male hormone in tablet form in women, *Brit. M. J.* **1:** 479–482 (March 23) 1940.
9. Twombly, G. H., and Millen, R. S.: Implantation of solid pellets of estrogens in treatment of menopausal symptoms, *Surg., Gynec. & Obst.* **72:** 605–610 (March) 1941.
10. Geist, S. H.; Walter, R. I., and Salmon, U. J.: Prophylactic implantation of estrogens following surgical and radium castration, *J. Mt. Sinai Hosp.* **8:** 543–536 (Jan.–Feb.) 1941.
11. Koenig, R.: Implantation sous-cutanée d'hormones oestrogènes, *Schweiz. med. Wchnschr.* **75:** 1065–1066 (Dec.) 1945.
12. Anselmino, J. K., and Schildbach, H. R.: Erfahrungen mit der therapeutischen Anwendung oestrogener Wirkstoffe in Kristallform, *Deutsche med. Wchnschr.* **72:** 179–183 (April 18) 1947.
13. Perloff, W. H.: The treatment of the menopause, *Am. J. Obst. & Gynec.* **58:** 684–694 (Oct.) 1949.

Endocrine Review

PREGNENOLONE

EDWARD HENDERSON, M.D., MARVIN WEINBERG, M.D.
AND W. ALLAN WRIGHT, M.D
From the Division of Clinical Research, Schering Corporation, Bloomfield, N. J.

PUBLICATIONS on the use of adrenocortical hormones in the treatment of rheumatoid arthritis and other collagen diseases have stimulated further research to determine the activity of steroids that have heretofore received little attention. Among those now being studied, pregnenolone appears to have certain advantages that should mark this compound as worthy of careful inquiry. It has an extremely low order of toxicity; it has not shown any adverse effect on endocrine physiology; and it has shown some promise in the treatment of rheumatoid arthritis.

A review of this interesting steroid seems timely.

Pregnenolone is a steroid, the 3-hydroxy analogue of progesterone, appearing in the penultimate stage of the oxidative preparation of progesterone (1, 2, 3). It has several physiologic actions which are not characteristic of steroid hormones of recognized groups, such as estrogens and androgens. Its chemical resemblance to progesterone has given rise to speculation about its interconvertibility, in vivo, according to a trend of thought often pursued at the present time, but it is seen that its actions are distinctive and are not readily traced to the formation of other steroids.

HISTORY

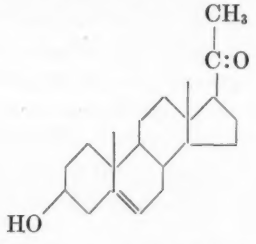
Pregnenolone was prepared chemically by Butenandt (4) in 1934, and was obtained by extraction from hog testes in 1943 by Ruzicka (5) and in 1948 by Kuizenga (6). Several investigators early tested it in animals for estrogenic (or anti-androgenic) (9), progestational and adrenocortical activity, but none was found in the range of dosage used (4, 7, 8, 9, 10). With considerably larger amounts, Selye (11) elicited a variety of effects in animals, the more important of which were a favorable influence on spermatogenesis and a protective action against damage to the testis induced by estradiol. Ruzicka (5) therefore felt that the presence of estrogen in the testis indicated an important role of pregnenolone. The first report of demonstrated physiologic activity in man was that of Hoagland (12), who found a reduction in the urinary excretion of 17-ketosteroids when this was elevated as a result of fatiguing activity. Davison and Koets (13)

Received for publication February 3, 1950.

utilized this effect, which they had observed in idiopathic hirsutism (14), as a clue to the management of ankylosing spondylarthritis (Marie-Strümpell), and obtained clinical benefit in that disease, as reported in 1949. During 1949 also, pregnenolone was intensively studied in the treatment of rheumatoid arthritis with results that were sufficiently encouraging to warrant further investigation. Considerable interest attaches to possible wider applications of this steroid because of its extraordinary apparent safety, its ready availability, and the early evidence of clinical usefulness.

CHEMISTRY

Pregnenolone is more correctly Δ^5-pregnene-3β-ol-20-one. It should be compared structurally with progesterone (the corresponding 3,20-dione) and should be distinguished from an isomer, Δ^4-pregnenolone-(20, 3), which has also been described (15, 16).[1] Though the trivial names are similar, pregnenolone should not be confused with pregneninolone, Δ^4-pregnene-20:21-ine-17β-ol-3-one, the orally-effective progestin known clinically as anhydrohydroxy-progesterone. The empiric formula is $C_{21}H_{32}O_2$, the structural formula is that shown here. The melting point is 190°C. (4,



18, 20–24), or 186–187°C. (25), or 192–194°C. (26); optical rotation is $[\alpha]_D^{26} = +35 \pm 4$ in absolute alcohol (6), $[\alpha]_D^{18} = +30 \pm 2$ in absolute alcohol (26). When crystallized from benzene or alcohol-acetone, pregnenolone occurs as white, opaque crystals without taste or odor. It is insoluble in water and difficultly soluble in most solvents except propylene glycol and mixtures of propylene glycol and alcohol. *Purity* is determined by melting point and optical rotation. *Identity* is determined by characterizing

[1] There is also a possibility of confusion with the 17-isomer which has been designated 17-isopregnenolene (17) and is defined as having the 17-alkyl group in alpha position. This isopregnenolone was produced by Butenandt in 1937: it is not precipitable with digitonin; it has m.p. 173°C., $[\alpha]_D = -140$ in alcohol (18). Pursuant to 1949 nomenclature (17), normal pregnenolone is the 17-β-alkyl compound, contrary to Selye's (19) older listing as a 17-α-isomer, compound No. 383, where 17-isopregnenolone is listed as a 17-β-isomer, compound No. 384. All data herein refer to normal pregnenolone, however otherwise designated by various authors.

pregnenolone either as itself; or as the acetate, m.p. 146–149.5°C., or 149–150°C. (26), $[\alpha]_D = +19.9$ or $+22 \pm 2$ in alcohol (18, 21, 26, 27); or the methyl ether, m.p. 123–124°C., $[\alpha]_D = +18$ in chloroform (7); or the 17-bromo-3-acetate (28, 29); or the oxide (30). *Color reactions* (Zimmermann) have been studied (31) and show generally lower absorption than with the 17- or 3-ketosteroids, with a maximum at 500 mμ, the curve for pregnenolone being distinctive. Other reactions using concentrated sulfuric acid, with and without benzaldehyde, have been recorded for the acetate (32). Data are available concerning the x-ray diffraction (33) and infrared absorption of pregnenolone (6). Pregnenolone was not found to reduce phosphomolybdic acid as in Heard's colorimetric determination. *Storage* of pregnenolone entails no difficulties, either in the dry form or in solution in propylene glycol or sesame oil: the compound is stable, and requires no special precautions.

TOXICITY

The acute toxicity of pregnenolone on oral administration to mice could not be ascertained, since doses up to 5 Gm. per Kg. of body weight were followed by no deaths, and no symptoms were produced.

The chronic toxicity of pregnenolone was studied in rats by (a) oral administration (in starch) of 1 Gm./Kg. three times weekly for 50 doses; (b) intraperitoneal injection (in saline suspension) of 0.2 Gm./Kg. three times weekly for 50 doses; and (c) oral administration in the food, 0.01 and 0.1 Gm./Kg., daily for three months. All three experiments failed to disclose a toxic action. In studies (a) and (b), no change was observed in the red blood cells, hemoglobin, white cell count or weight of viscera, and in (c) there was no difference in growth rate, food intake, fertility, or size and condition of litters, when the treated animals were compared with controls (34). Male and female albino rats receiving 0.01 Gm./Kg. subcutaneously daily for three months showed no change in the weight of the several viscera (35).

Toxicity in man. Some appraisal of the toxicity of pregnenolone for man can be obtained from various clinical reports. The study of Tyler and others (36) on the effect upon oligospermia in 25 men receiving oral dosage of 25 to 75 mg. daily and intramuscular dosage of 5 to 20 mg. of pregnenolone or pregnenolone acetate revealed a side action in one patient only:

> "One patient was started on 50 mg. per day orally, but after a week had a diffuse erythematous skin eruption which he had not experienced previously, and the medication was discontinued, following which the eruption disappeared; thus it must be assumed that this was a mild toxic reaction. No other significant untoward reactions were reported to us by the patients." (36)

Davison and co-workers (37) report that 8 patients received 50 to 150 mg. pregnenolone acetate in oil, daily, by intramuscular injection, without adverse effect. Although this group at first withheld judgment of possible later effects, stating

> "We do not recommend the administration of pregnenolone until prolonged administration shows no deleterious effects on other hormonal activities" (13),

subsequent communications revealed no toxic action apparent in the further course of these patients, including treatment with 100 mg. daily for as long as seventy-five days. No effect on the regularity of the menstrual cycles or on the character of menstruation was observed in 2 hirsute patients after oral administration of 50 mg. daily for three months, although the ketosteroid excretion in these patients dropped markedly to within normal limits (38). Davis (39) observed no side effects of any kind in 10 patients receiving 100 to 300 mg. of pregnenolone in aqueous suspension, daily, for five weeks.

Thus far, the evidence suggests an absence of toxic actions in man, following parenteral administration of pregnenolone, comparable with the experience in animals. The single instance of erythema on oral administration of 50 mg. daily is interesting in view of the large experience of the Worcester group (12, 40–42) with oral doses of 25 to 100 mg. daily without side effects of any kind:

> "Finally we should like to point out that we have encountered no deleterious results in connection with the ingestion of Δ^5-pregnenolone in our studies involving several hundred men and women who have taken the medication; in some instances in doses of 100 mgm. per day for as long as four months. The substance is nontoxic." (41)

The possibility of side actions due to the prolonged parenteral administration of pregnenolone in large doses cannot, of course, be excluded. One anticipates, however, that any such side actions are likely to reflect alterations in physiology, rather than forthright toxicity as in the case of pharmacologic compounds. None of the known activities seems to suggest a disturbance to be anticipated with intensive use, save the peculiar anesthetic action of all steroid hormones in massive dosage (43, 44).

PHARMACOLOGY

Pregnenolone has few pharmacologic actions, if any, which are not of a hormonal character: such as effects on the pituitary and sex organs, and interactions with other hormones. In an examination of the *anticonvulsant action* of numerous steroids in animals receiving electric shock, pregnenolone was found (45) to have negligible activity; and subcutaneous doses of 0.005 Gm./Kg. did not influence picrotoxin convulsions in mice (34): *i.e.*, the effect of pregnenolone was less apparent than that of propylene

glycol used as the solvent. However, an anticonvulsant action against metrazol and against electrically induced convulsions in the cat has been described (46). *Blood chlorides* were persistently raised by pregnenolone and by estrogens, according to a report of Selye and Dosne, cited (ref. no. 197) by Selye (11). Tissue *potassium* (of brain and muscle) was unaffected in normal rats by the administration of "small" amounts of pregnenolone, 0.0035 Gm./Kg., but was reduced in adrenalectomized rats (47). *Nitrogen balance* was only slightly or not significantly improved by dosage as low as 30 mg. per day, intramuscularly, for eighteen days, in a patient with postmenopausal osteoporosis (48)—even a more pronounced gain in phosphorus balance in the same or a similar patient being termed "inconclusive" (49)—and the nitrogen balance could not be maintained in Cushing's syndrome (50). In one person the administration of 100 mg. daily for thirty-six days was described as leading to an increase in plasma proteins, but the circumstances of the study (route of administration, underlying diagnosis, effect on nitrogen balance) are not disclosed (51).

HORMONAL ACTIONS

A large volume of experimentation has been carried out by Selye and other workers, consisting of exceedingly high dosage of pregnenolone administered to animals, chiefly the rat. The findings are summarized for their possible theoretic value, although it is notable to what little extent they are reflected in human clinical experience.

1. Pituitary

Pregnenolone does not prevent the appearance of castration cells in immature female rats (52), nor affect the increase in weight of the pituitary of adult male rats receiving very large doses of estradiol, 0.001 Gm./Kg. (53). In adult female rats which are castrated *and* hypophysectomized, the administration of a pituitary extract produces a greater weight of the preputial glands if pregnenolone also is given. The difference due to pregnenolone has been called a "synergistic" action with pituitary extract (25). This could not be confirmed (54) in hypophysectomized animals, but a greater effect of prolan on uterine weight and luteinization was found when pregnenolone was used in intact animals.

2. Testis

After hypophysectomy in male rats, pregnenolone maintains the weight of the testes and maintains spermatogenic activity. The seminiferous tubules are large, spermatozoa are numerous (although some desquamation is seen), and the testes weigh 0.6 to 0.7 times as much as in the

intact animals. The effect does not extend to the interstitial tissue, however; this is atrophic, and the seminal vesicles are reduced to 0.2 times normal weight (55). The findings of Albert and Selye (56) are thus confirmed. The experimental situation is held to afford a test of what has been termed a "gonadotropic" activity of steroids. That of pregnenolone is perceptible, but it compares with that of testosterone as only 1:50, as shown by data of Simpson (57): Hypophysectomized male rats received daily doses of pregnenolone or testosterone propionate for fifteen days, with the following results:

Compound	*Daily dosage*	
Pregnenolone	2.5 mg.	Increased testis weight
Pregnenolone	5.0 mg.	Increased testis weight and spermatozoa
Testosterone	0.1 mg.	Increased testis weight and spermatozoa

Pregnenolone did not maintain spermatogenesis in artificially cryptorchid rats (58). In immature hypophysectomized animals, as Masson (59) has shown, pregnenolone readily maintains testis weight at 20 or 4 mg./Kg., but the lower dosage fails to sustain spermatogenesis. More recently (60), the distinction is made between "active spermatogenic" agents such as testosterone, which restore the weight of testes after hypophysectomy,[2] and "passive spermatogenic" agents such as pregnenolone, which merely maintain the existing weight. The data are asserted to provide a "basis for the rational selection" of therapeutic steroids, although experimental dosage of 0.03 Gm./Kg. of testosterone and 0.05 Gm./Kg. of pregnenolone, corresponding to 2 and 10 Gm. per day clinically, was used. Reference is made, later, to failure to improve clinical oligospermia with oral dosage up to 75 mg. pregnenolone daily, or parenteral dosage up to 20 mg. daily (36). Further, 50 mg. pregnenolone was administered daily by intramuscular injection for periods of one and three months by Heller (61) without effect in oligospermia. He noted no untoward reaction. It may not be worth while to conjecture that 200 to 500 times as much pregnenolone might have given a better result.

3. Secondary sex organs.

Pregnenolone, like progesterone, fails to maintain the weight of the seminal vesicles in castrated adult rats, or hypophysectomized adult rats (55), or hypophysectomized immature rats (59); but the preputial glands of castrated female rats are enlarged (25), especially if the adrenals are intact,

[2] Gasche and Schuler (54) state that neither testosterone nor pregnenolone can restore the testis once it is atrophied, but they refer to no explicit experimental result or dosage.

whereas those of males are little affected (62). Pregnenolone, like progesterone, produces enlargement of the prostate of immature rats (25) if a dosage of 0.2 Gm./Kg. is used. Although androgens, of course, restore the weight of accessory glands in hypophysectomized animals, pregnenolone merely retards the decline in weight after hypophysectomy (54). The uterus of rats, after castration, is enlarged by pregnenolone (63), depending on the dosage used (52). The enlargement is less after hypophysectomy (25). The vagina is continuously cornified (64), as with estrogens, androstenediol and dehydroepiandrosterone. Cornification is produced also in hypophysectomized rats, and uterine weight is increased, using 0.012 Gm./Kg. daily, but only if the ovaries are present (65). Gasche and Schuler (54) agree that pregnenolone is estrogenic, provided the dosage is 5 to 10 times that stated by Selye (65), but then go on to say that specially purified pregnenolone is nc longer estrogenic. If estrogenic activity is exerted in man, it has certainly not been detectable in the dosage range employed thus far. Finally, it is of interest also in this connection that in the castrated rabbit estrogens produce a uterine hyperemia which can be prevented by atropine and which is traced to an increased concentration of acetylcholine (66), and that not only estrogens but also pregnenolone increases the production of acetylcholine by a suspension of frog brain tissue (67).

4. Pregnenolone and other hormones.

(*a*) *Progesterone.* The residual structure, the "metrial gland," following the production of artificial deciduoma in the rat, is maintained by progestins and therefore serves as a test object. Pregnenolone was not found to antagonize this action of progesterone (68). Indeed, by McPhail's rabbit test, pregnenolone appeared to be itself a progestin of low activity: 1.0 i.u. was represented by 40 to 100 mg. (69).

(*b*) *Androgens.* Neither local nor systemic antagonism to androgens is found in the capon test (9). Clinically, however, there is some as yet ill-defined implication that the virilizing action of testosterone in women is modified by pregnenolone. The "atrophy" of the testis seen after "small" doses of testosterone in rats, 1 mg. daily in animals weighing 121 Gm., is counteracted by 10 times the dose of pregnenolone (70) (*see* also subsequent section No. 6, Kidney).

(*c*) *Estrogens.* Under certain conditions, in animals, estradiol produces a decline in the weight of the testis: the action is offset by pregnenolone (53, 54). For example, the testes of rats are maintained about equally well if either 2 or 10 mg. pregnenolone be given concurrently wth 300 micrograms of estradiol daily (53); but the testes and "adnexae" are not restored by

pregnenolone (54). Pregnenolone has been given clinically as part of the treatment of a man who accidentally received estrogenic overdosage; but it is not possible to distinguish what effects it may have had (71).

(*d*) *Gonadotropins.* The data mentioned under Pituitary, and also the reported antagonism to estrogen and androgen, should be reconsidered. Some of these effects evidently depend on the presence of gonadal tissue (62). For example, estradiol reduces the weight of the seminal vesicles, while pregnenolone given concurrently maintains them at somewhat less than normal weight ("elicits a marked hypertrophy") (53); but the action is not found if either castrated adult rats or immature animals are used (62). Although pregnenolone itself does not affect the preputial glands of hypophysectomized adult female rats, it exerts a "synergistic" action with pituitary extract (25) prepared in a certain way. These studies are complicated by want of a sufficiently isolated test object, but it is readily inferred that a semblance of gonadotropic action is obtained with pregnenolone, much as with testosterone, under suitable experimental conditions.

5. Anesthetic action.

In intact rats, up to 50 mg. of pregnenolone is not anesthetic (72), but anesthetic action is present in partially hepatectomized rats (43, 44) and in minnows (44), using 12 mg. Compared with other steroids showing this activity, pregnenolone is only moderately active.

6. Kidney.

Pregnenolone produces a slight increase in kidney weight in hypophysectomized female rats (25). In castrated male rats, the implantation of pregnenolone in the form of a pellet results in diminution of kidney weight without change in the relative concentration of alkaline or acid phosphatase (73). Pregnenolone has a variable effect on the aldehyde staining properties of the kidney of castrated female rats, under circumstances in which estrogens and progestins deplete tissue-aldehyde and androgens prevent the depletion (74). One normal man receiving 100 mg. of pregnenolone daily, parenterally, for thirty days, did not show alteration in glomerular filtration, renal blood flow, or tubule secretory mass (75). Given in undetermined dosage in man, pregnenolone does not alter clearance of mannitol or *p*-aminohippuric acid, nor glucose or PAH "T_m" (76).

7. Adrenocortical activity.

By various criteria, it has been shown that pregnenolone has cortical activity (43), as in maintaining the life of adrenalectomized rats (77, 78); the action is described as similar to that of desoxycorticosterone, but of lower intensity (79). However, the lymphocyte count is not reduced in

rats (47), glucose tolerance is unchanged in man (47), and the protective action of desoxycorticosterone against damage by x-ray in animals, as computed from the increase in the lethal dose of x-ray administered in a specified manner, is not found with pregnenolone (80). One explanation of the role of pregnenolone in improving fatigue and stressed performance (12, 40–42) is that it might "spare" adrenocortical activity, which is shown to be exaggerated (81–83) during such effort.

8. Others.

Pregnenolone is reported to protect guinea pigs against the production of fibroma (84). It is described as having some lactogenic effect similar to that of prolactin (85).

These diverse data make it possible to ascribe a variety of the classic hormonal actions to pregnenolone, but it should be recognized that the actions are elicited only under unusual experimental conditions and at high dosage, and even then are not of outstanding intensity. Hence it may be confusing, at best, to view this substance as a "gonad-protecting" (53, 56) or "gonadotropic" (53) hormone or as another "male sex hormone" (5). That such a variety of the customary hormonal actions can be elicited in some degree with a substance that appears actually to be devoid of all of them would imply that such experiments as have been cited do not serve to screen a compound, the action of which does not fall within any pattern thus far recognized.[3] Likewise, the conception that pregnenolone operates by being first converted into some more familiar steroid hormone (12) is not well borne out by the findings to date: the extent of conversion would need to be meager, and the end-product somewhat different in each experiment.

CLINICAL

Several clinical applications of pregnenolone have been studied, on an empiric basis, and have provided results of some interest. An early applica-

[3] If pregnenolone has indeed a physiologic role as such, comparable to the major steroid categories exemplified by androgens and estrogens, it is handicapped in its history, so far, because the material is better known than its possible role. This is the reverse of the state of affairs in which androgens, for example, were quite well understood as a distinctive physiologic class, even before the first compounds exemplifying the activity were isolated. It was then relatively easy to identify and compare successive androgens as such. But if androsterone were isolated before androgenic function was understood, it is likely that an extensive literature would have described its antagonism to estrogens, and speculated upon its possible conversion to thyroid, etc., in order to account for the observable actions in familiar terms. The necessary physiologic datum being supplied, these efforts would have become unnecessary, and their fruitlessness apparent. Perhaps it will so come to pass with pregnenolone.

tion was related to fatigue and endurance; some effort was devoted to the problem of spermatogenesis; and most recently the compound has been administered in various forms of arthritis.

1. Fatigue and endurance.

Hoagland (12) showed that fatigue, produced by a standardized method which simulated the operation of an airplane in flight, was objectively measurable in terms of the urinary elimination of 17-ketosteroids. The latter could be correlated with the number of errors made in tests, with time actually spent in the air, or with anoxia, in that the more stressed activity was reflected in a higher excretion of 17-ketosteroids. Fatigue was found to induce an increase in 17-ketosteroid excretion which could be plotted on a line of given slope. When pregnenolone, 50 mg. daily by mouth, was administered, the slope of this plot was reduced to one half its original value. Adrenal cortical extracts and progesterone were not found to have this effect. Pregnenolone was further described as improving the scores obtained in test runs, and as leading to a diminution in the feeling of fatigue.

Pincus and Hoagland (40) trained 5 college students to constant performance on a fatiguing-machine, and then studied the effect of oral adrenal cortical hormone, progesterone by injection, and pregnenolone by mouth. In three-hour runs, only pregnenolone appeared to show a significant influence on the scores. Using a target meter (an automatically-scoring device operated like the stick of an airplane), 14 subjects were tested under treatment with 50 mg. of pregnenolone per day, orally, in an elaborate scheme of deceptions calculated to rule out suggestibility. It was concluded from the scores that pregnenolone had a cumulative effect during two weeks of administration, persisting several days afterward, consisting of an obvious improvement in performance. Seven of the subjects were pilots concurrently engaged in active flying: some of these volunteered the information that they felt better in their work, and some appeared able to distinguish positively when the placebo was being substituted.

These investigators (41, 42) then administered 25 mg. to 75 mg. of pregnenolone daily by mouth to 8 leather cutters, 12 lathe operators, and 77 optical workers, using as criteria of effect their productivity, wastage of material and perfection of the finished product. Some subjective responses were noted, consisting of a feeling of well-being, a tendency to tire less easily, and a sense of being better able to cope with the job. As to productivity, no effect was seen when the work was unhurried and unstressed (*e. g.*, leather cutting) or when the worker had little enthusiasm for what he was doing (*e. g.*, young men about to be conscripted); but in 2 groups of optical workers, a clear-cut improvement was evident with 75 mg., less with 25 mg., and none with the placebo. The effect outlasted the treatment.

The least noticeable effect was observed in a department working at leisure, *i.e.*, not "under pressure."

Forbes, with Graham-Bryce and Bullen (86), tested 6 persons on a target meter after the administration of 50 mg. of pregnenolone daily, without observing any significant effect. Every effort was made to confirm any aspect of the previous findings (40), but without success. Remarkable consideration was given to the discrepancy, which was attributed in part to the absence of stress in the performance of the test subjects; personal motivation was recognized as an important variable, such that seemingly comparable studies might yield distinctly opposed results if one group felt obliged to succeed, while the other merely performed the work.

The relation of steroid hormones to endurance in animals, especially in race horses, has been studied (87); some improvement in performance has apparently been traced to pregnenolone.

2. Myasthenia gravis.

Six patients obtained no benefit from pregnenolone (88).

3. Spermatogenesis.

In 25 patients having oligospermia (less than 60 million sperms per cc.), poor motility, or high proportions of abnormal sperms, pregnenolone was given orally or intramuscularly for periods up to twenty weeks, using in most instances 50 mg. daily by mouth, or 10 to 20 mg. daily intramuscularly, of either pregnenolone or its acetate. Although in a few cases there may have been improved motility, and at least 1 patient had a higher sperm count after treatment, the results, on the whole, with these dosages, were not considered to be of any significance (36).

4. Arthritis.

Lansbury (89) was the first to suggest the use of pregnenolone in the treatment of *rheumatoid arthritis* in December 1946, and gave three reasons for concluding that it might be useful:

"1. The well marked and often prolonged fatigue which precedes and accompanies the disease.

"2. The recently established relationship between the adrenal cortex and the immunity mechanism of the body.

"3. Some data which I have obtained on patients by using crude extracts of the whole adrenal cortex."

In March of 1947 he reported the following effects of the oral administration of pregnenolone:

"In three cases of rheumatoid arthritis there was subjective improvement with less fatigue, a gain in strength, less pain, and in two cases an actual measurable reduction in swelling. In one case there was relief of a constant dull pain in the eyes which had been

present for many months and a relief of nervousness and an increased ability to sleep so that sedation could be discontinued. In considering these effects I feel that there is a suggestive lead here. There is no evidence that pregnenolone is curative but I think further trial is justified. It seems to me, as I think over the cases, that the fundamental thing which has occurred is a lessening of swelling possibly due to alteration of permeability of the cell membranes. If this is true, then pregnenolone might be of use in many conditions where swelling or slowed up transmission of fluid across membranes is a factor. I found pregnenolone to be of no use in one case of osteo arthritis and one case of spondylitis, but naturally this trial is not sufficient to make a final evaluation in these diseases."

Following Lansbury's results, further investigations were carried out, using pregnenolone by mouth, and the evidence indicated that sufficient dosage, of the order of 1 Gm. per day orally, might have the effects desired.

In 1949, Davison and Koets (13) reported favorable effects of pregnenolone by injection in *ankylosing spondylarthritis*. These investigators reported (90) that whereas in rheumatoid arthritis the urinary 17-ketosteroids were normal, they were elevated in spondylarthritis. As pregnenolone had been shown (12, 14) to reduce the excretion of 17-ketosteroids, these authors (13) administered pregnenolone acetate in oil in a dosage of 50 to 150 mg. daily, intramuscularly, for varying periods. In 7 male patients and in 1 female patient they found a reduction in urinary 17-ketosteroids, but, in addition, there was a striking relief of subjective and objective evidences of arthritis, including pain, muscle spasm, and limitation of motion. Improvement was usually well marked in patients receiving 100 mg. of pregnenolone acetate in oil by intramuscular injection daily, and in the female patient equalled that found in the males. In a recent publication these investigators (91) have contributed valuable additional details of this experience.

In *arthritis complicating prostatitis*, 5 patients receiving 50 mg. of pregnenolone intramuscularly, at the same time as 500 mg. of sodium ascorbate intravenously, experienced marked relief of pain within a few minutes, followed by greatly increased mobility of the affected joint (shoulder, hip, knee). The treatment was repeated one to several days later, as required (92).

Rheumatoid arthritis. The following are briefly summarized reports of therapeutic trials with parenteral pregnenolone, of which we have detailed knowledge. Although it will be seen that, in general, the compound has apparently provided benefit to about one half of the patients treated, one group of investigators (93) has reported that it had *no effect whatsoever*, either subjective or objective. Reports of this nature are so at variance with the experience of other groups of workers as to make it obvious that an absolute evaluation of the role of pregnenolone in rheumatoid arthritis is not easy. One difficulty may lie in the existence of sub-types of the disease,

which can be distinguished by their response to steroid therapy; another, in possible variations in the patient's physiology or nutrition which enable a response to be achieved at one time and not at another; still another, scarcely incidental factor is the profound psychologic effect upon patients of recent widely publicized sensations and half-truths about "miraculous results" to be obtained with hormones, by now so marked that an exceptional response can be expected following the injection even of normal saline solution. This is in keeping with the observations of rheumatologists of long experience, who have found that these long-suffering patients frequently appear to benefit from *any* "new form of treatment."

One is therefore inclined to discount all excessively favorable reports, no matter how impressive at face, and to give consideration only to objective evidences of improvement. There is no sharp line of demarcation, however, between a patient's subjective idea or relief of stiffness and the attending objective increase in range of motion. Moreover, there is no helpful recourse to laboratory findings, since obvious changes in the disease may be unaccompanied by a change in the sedimentation rate; and other determinations, such as might be specific for the disease process, are not known to us thus far. Hence we reach the position that it is impossible to reconcile differing results, and injudicious to form sanguine estimates or claims for the effect of pregnenolone. Some patients have responded well; and there is no doubt whatever as to the response. It is therefore proper to conclude that pregnenolone is a useful tool for the study of rheumatoid arthritis, and that it is by all means worth investigating further.

The several reports of therapeutic trials are here presented for consideration in this light.

Davis (39) has administered from 50 to 300 mg. of pregnenolone daily, by intramuscular injection, obtaining no response whatever in a small proportion of cases but in others noting definite symptomatic and objective improvement. Thus, of the first 17 patients treated, 3 were unaffected, 9 showed clinical benefit graded+ or ++, and 5 improved to +++ or ++++ degree. The sedimentation rate was uniformly unaffected by pregnenolone treatment in this series of cases. There was a general relationship between dosage and response in that the patients treated for the longest time and with the highest dosage appeared to show the best clinical results.

Cohen (94) in 1949 described improvement in about 75 per cent of patients receiving large doses of pregnenolone parenterally. In the first series (95) of 20 patients, treated with total dosage up to 12,000 mg. during six months, benefit was well marked in 12 patients, and slight in 3, while 2 were unimproved, 1 received only a single injection, and 2 had adverse reactions consisting of swelling of the hands and lips, with urticaria, and

the production of an abscess at the site of injection, respectively. "Several patients responded to therapy immediately and dramatically. Uniformly, swelling and pain were relieved," and long-standing disabilities cleared up. A usual dosage of 300 mg. daily was adopted, and was found to cause no untoward reaction in any except the allergic patient, "which was probably due to the menstruum."

Cohen (96) has subsequently reported experience with additional patients, without material change in the rate of good response, and has contrasted the absence of adverse effects, using 300 mg. of pregnenolone daily for upwards of forty days, with the findings in patients receiving some other new substances. The nature of the result is described in some detail:

"... the first improvement noted was absence of pain on motion and disappearance of tenderness. Absence of pain on motion permits greater joint mobility which in turn leads to less spasticity and greater muscular strength. The patient who responds to pregnenolone therapy is usually able within a few days to perform muscular actions impossible for previous months, or even years, such as dressing, stooping to tie shoes, combing hair, sitting and rising, lying and rising, raising arms above the head and lowering them slowly ... climbing and descending stairs. ..."

Warter (97) found, in a series of 21 patients, that 5 were unimproved and 16 were benefited as follows:

slight improvement:	4 patients, or 19 per cent
moderate improvement:	4 patients, or 19 per cent
marked improvement:	8 patients, or 38 per cent

the dosage being 100 mg. of pregnenolone acetate, daily, for five to thirty days, followed by 100 mg. one to three times weekly, all by intramuscular injection. All except 2 of the patients who showed benefit initially continued to benefit by further treatment. In 9 patients in whom uric acid excretion was determined, an increase was reported in the four-hour period after injection in 7, and an insignificant decrease in 2. Urinary creatinine was described as having declined during the same period in a majority of cases, so that the ratio *uric acid: creatinine* usually increased. Over a two-month period, the excretion of creatinine was said to be reduced, implying a diminution in tissue necrosis presumably about the affected joints. However, these findings have not been confirmed.

Ishmael, Hellbaum, Kuhn and Duffy (98) employed pregnenolone in doses up to 100 mg. daily, alone and as adjuvant to a schedule of high-level combined androgen and estrogen therapy, in a group of 123 patients. Considerable individual variation was encountered; some patients responded better to the combination pregnenolone-testosterone than to either alone. "Approximately 50 per cent of the patients with rheumatoid arthritis treated with pregnenolone responded quite satisfactorily" (99).

Davison, Koets, Snow and Gabrielsen (91) have reported the treatment of 30 arthritic patients with pregnenolone, including 14 with rheumatoid arthritis who were given up to 200 mg. daily of pregnenolone acetate in oil solution or 300 mg. daily of pregnenolone in aqueous suspension by intramuscular injection for periods of three to eight weeks, the active material being used alternately with placebos in some patients. The study continues in progress. In those having arthritis affecting the extremities, there were found relief of stiffness and pain, great improvement in muscle strength, relief of fatigue, and a feeling of well-being, "usually within a week and sometimes within three or four days." Reduction in joint swelling, especially if there were large effusions, was slow, and reduction of the sedimentation rate, if any, did not parallel clinical improvement but followed it. No toxic action was noted; sexual potency and the menstrual cycle were not affected. All patients experienced a definite feelingof well-being, but there was no sign of euphoria to the point of exhilaration save in 1 patient, who had difficulty in sleeping while receiving the maximum dosage. Symptoms recurred shortly (*e. g.* five days) after treatment was discontinued. *Other forms* of arthritis treated in the same study,without benefit, were rheumatoid arthritis in a patient with psoriasis, severe gouty arthritis in one patient, and disseminated lupus in a girl who was too emaciated to continue treatment, although some improvement had been obtained in the joint symptoms and swelling.

Manning (100) felt that the clinical actions of pregnenolone in rheumatoid arthritis were largely subjective, with less stiffness and pain and in some instances increased movement of the joints. He found small and variable changes in 17-ketosteroid excretion, but found slight to marked increase in urinary pregnanediol; the blood eosinophil count was reduced after initial treatment, but later practically all the patients showed marked increases. As in other studies, there was no regular change in the sedimentation rate. Toxic reactions were not encountered. In later communications Manning and Brugsch (101) have reported that they are now convinced pregnenolone has no useful function in the treatment of rheumatoid arthritis, and they question whether those patients who do not respond have "true" rheumatoid arthritis. This may be an expression of possible subtypes of the disease, as mentioned before.

Clinically, it is apparent that some patients who eventually respond well may not exhibit any change at all during the first few weeks of treatment. It seems advisable, therefore, for treatment to be continued for three to four weeks before final evaluation is attempted in any one patient.

Whether the effects thus described in the arthritides are purely palliative or embody fundamental changes in the physiology of the disease must be determined by further experience. That the sedimentation rate, a time-

honored reflection of the essential disease process, is not uniformly affected appears to show that the action is symptomatic only, but does not exclude intercession in actual cellular processes. Doubtless other determinable chemical actions will be recognized, and the nature of the steroid role finally characterized. In any event, the deficiency of data of this kind in the treatment of arthritis is not limited to pregnenolone.

SUMMARY

Pregnenolone is a steroid having pharmacologic and hormonal behavior which has not been satisfactorily characterized within any familiar framework. A fair proportion of rheumatoid arthritics receiving pregnenolone in sufficient dosage, for an adequate period, show symptomatic changes sufficiently favorable to warrant further study. The substance has an extraordinarily low order of toxicity. Its properties are diverse, and somewhat obscure, and seem intriguing enough to encourage further inquiry.

Addendum

Since this manuscript was prepared we have had communications from a number of investigators to the effect that when 300 mg. per day failed to induce a favorable response in rheumatoid arthritis, doses of 400 mg., 500 mg., and even 600 mg. of pregnenolone per day were given and were followed by clinical benefit in some of the patients. Thus it is not possible at this time to establish the upper limit of useful dosage. It appears, moreover, that very large doses can be employed without danger of side-effects.

REFERENCES

1. Fernholz, E.: Zur Synthese des Corpus-luteum-Hormons, *Ber. d. deutsch. chem. Gesellsch.* **67**B: 1855, 1934.
2. Oppenauer, R. V.: Eine Methode der Dehydrierung von sekundären Alkoholen zu Ketonen. I. Zuer Herstellung von Sterinketonen und Sexualhormonen, *Rec. d. trav. chim. d. Pays-Bas* **56**: 137–144, 1937.
3. Ruschig, H.: Halbsynthese des Progesterone mit Hilfe des Curtiusschen Abbaues, *Med. u. Chem.* **4**: 327–342, 1942.
4. Butenandt, A.; Westphal, U., and Cobler, H.: Über einen Abbau des Stigmaterias zu Corpus-luteum-wirksamen Stoffen; ein Beitrag zur Konstitution des Corpus-luteum-Hormons, *Ber. d. deutsch. chem. Gesellsch.* **67**B: 1611–1616, 1934.
5. Ruzicka, L., and Prelog, V.: Untersuchungen von Extrakten aus Testes. Zur Kenntnis der Lipoide aus Schweinstestes, *Helvet. chim. acta.* **26**: 975–983, 1943.
6. Haines, W.; Johnson, R. H.; Goodwin, M. A., and Kuizenga, M. H.: Biochemical studies on hog testicular extract. I. Isolation and identification of 5-pregnen-3-(B)-ol-20-one, *J. Biol. Chem.* **174**: 925–937, 1948.
7. Butenandt, A.: Recherches chimiques sur la spécificité physiologique du groupe des hormones génitales, *Bull. Soc. chim. biol.* **19**: 1477–1497, 1937.
8. Mühlbock, O.: Publication unavailable. Cited by Selye (25) as: Internat. cong. verlosk. en gyn. (Amsterdam) **4–8**V: 491, 1938.

9. MÜHLBOCK, O.: The antagonism between the action of the comb-growth substances on the one hand and the estrogenic substances and progesterone on the other, *Acta brev. Neerland* **8**: 50–52, 1938.
10. SEGALOFF, A., and NELSON, W. O.: Action of some synthetic steroids in adrenalectomized immature rat, *Endocrinology* **31**: 592–596, 1942.
11. SELYE, H.: The pharmacology of steroid hormones and their derivatives, *Rev. canad. de biol.* **1**: 577, 1942.
12. HOAGLAND, H.: Adventures in biological engineering, *Science* **100**: 63–67, 1944.
13. DAVISON, R. A., and KOETS, P.: The effect of Δ^5-pregnenolone on urinary 17-ketosteroids and symptomatology of ankylosing spondylarthritis. Read before the International Congress of Rheumatic Diseases, New York, May, 1949.
14. KOETS, P.: Personal communication, February, 1949.
15. BUTENANDT, A., and SCHMIDT, J.: Über Δ^4-Pregnenol-(20)-on-(3); ein Beitrag zur Specifizät der Corpus-luteum-Wirkung, *Ber. d. deutsch. chem. Gesellsch.* **67**: 2092–2094, 1934.
16. MARKER, R. E.; CROOKS, H. M., JR., and WITTBECKER, E. L.: The action of selenious acid on Δ^4-pregnenediol and on Δ^5-androstenediol, *J. Am. Chem. Soc.* **63**: 777–779, 1941.
17. FIESER, L. F., and FIESER, M.: Natural Products Related to Phenanthrene, ed. 3, New York, Reinhold Publishing Corporation, 1949, p. 443.
18. BUTENANDT, A., and FLEISCHER, G.: Über 17-iso-Δ^5-Pregnenol-(3)-on-(20), *Ber. d. deutsch. chem. Gesellsch.* **70**: 96–102, 1937.
19. SELYE, H.: Encyclopedia of Endocrinology. Sec. I. Classified Index of the Steroid Hormones and Related Compounds, Montreal, A. W. T. Franks Publishing Company, 1943, pp. 383, 384.
20. ALLEN, E., editor: Sex and Internal Secretions, Baltimore, Williams and Wilkins Company, 1939, p. 907.
21. BUTENANDT, A., and SCHMIDT-THOMÉ, J.: Überführung von Dehydroandrosteron in Progesteron: ein einfacher Weg zur künstlichen Darstellung des Schwangerschaftshormons aus Cholesterin, *Ber. d. deutsch. chem. Gesellsch.* **72**: 182, 1939.
22. BUTENANDT, A., and WESTPHAL, U.: Einige Erganzungen zur Chemie des Gelbkorper-Hormons, *Ber. d. deutsch. chem. Gesellsch.* **69**: 443–447, 1936.
23. REICHSTEIN, T., and SCHINDLER, W.: Über Bestandteile der Nebennierenrinde und verwandte Stoffe, *Helvet. chim. acta* **23**: 669, 1940.
24. MARKER, R. E., and KRUEGER, J.: Sterols, CXII. Sapogenins, XLI. The preparation of trillin and its conversion to progesterone, *J. Am. Chem. Soc.* **62**: 3349–3350, 1940.
25. SELYE, H., and CLARKE, E.: Potentiation of a pituitary extract with Δ^5-pregnenolone and additional observations concerning the influence of various organs on steroid metabolism, *Rev. canad. de biol.* **2**: 319–328, 1943.
26. WETTSTEIN, A.: Über Steroide. Homologe der Keimdrüsenhormone IV. Höhere Homologe des Pregnenolons und Progesterons, *Helvet. chim. acta* **23**: 1371–1379, 1940.
27. MARKER, R. E.; CROOKS, H. M., JR.; JONES, E. M., and SHABICA, A. C.: Sterols, CXLIII. The conversion of 5-pregnen-3-(β)-ol-20-one to dehydro-iso-androsterone, *J. Am. Chem. Soc.* **64**: 1276–1280, 1942.
28. HEGNER, P., and REICHSTEIN, T.: Über Bestandteile der Nebennierenrinde und verwandte Stoffe, vereinfachte Teilsynthese von Substanz L. und Pregnen (5) diol-3β, 17β-on-(20), *Helvet. chim. acta* **24**: 828–832, 1941.

29. Ruzicka, L., and Meldahl, H. F.: Über Steroide und sexual Hormone, *Helvet. chim. acta* **22**: 421–424, 1939.
30. Ehrenstein, M., and Stevens, T. D.: Investigations on steroids. VI. New method of preparing 6(a)-acetoxy-progesterone, *J. Org. Chem.* **6**: 908–919, 1941.
31. McCullagh, D. R.; Schneider, I., and Emery, F.: Spectrophotometric studies of the m-dinitrobenzene reaction with sex steroids, *Endocrinology* **27**: 71–78, 1940.
32. Scherrer, I.: Über Farbreaktionen des Benzaldehyds mit Sterinen und Steroiden beim Unterschichten mit konz. Schwefelsäure, *Helvet. chim. acta* **22**: 1329–1340, 1939.
33. Spiegel-Adolf, M., and Henny, G. C.: X-Ray Diffraction Studies in Biology and Medicine, New York, Grune and Stratton, 1947, pp. 174–177.
34. Tislow, R.: Unpublished data.
35. Selye, H., and Beland, E.: The development and repair of organ changes induced by steroid compounds, *Rev. canad. de biol.* **2**: 271–289, 1943.
36. Tyler, E. T.; Payne, S., and Kirsch, H.: Pregnenolone in male infertility, *West. J. Surg.* **56**: 459–463, 1943.
37. Davison, R. A.: Personal communication.
38. Koets, P.: Personal communication, July, 1949.
39. Davis, J. S., Jr.: Personal communication, July, 1949.
40. Pincus, G., and Hoagland, H.: Effects of administered pregnenolone on fatiguing psychomotor performance, *J. Aviation Med.* **15**: 98–115, 1944.
41. Pincus, G., and Hoagland, H.: Effects on industrial production of the admininstration of Δ^5 pregnenolone to factory workers. I., *Psychosom. Med.* **7**: 342–346, 1945.
42. Pincus, Gregory; Hoagland, Hudson; Wilson, C. H., and Fay, N. J.: Effects on industrial production of the administration of Δ^5 pregnenolone to factory workers, II., *Psychosom. Med.* **7**: 347–352, 1945.
43. Selye, H.: Correlations between the chemical structure and the pharmacological actions of the steroids, *Endocrinology* **30**: 437–453, 1942.
44. Selye, H., and Heard, R. D. H.: Fish assay for anesthetic effect of steroids, *Anesthesiology* **4**: 36–47, 1943.
45. Spiegel, E., and Wycis, H.: Anticonvulsant effects of steroids, *J. Lab. & Clin. Med.* **30**: 947–952, 1945.
46. Spiegel, E.: Personal communication, May, 1946; March, 1950.
47. Hoagland, H., and Stone, D.: Brain and muscle potassium in relation to stressful activities and adrenal cortex function, *Am. J. Physiol.* **152**: 423–435, 1948.
48. Reifenstein, E. C., Jr.: The Protein Anabolic Activity of Steroid Compounds. Supplement to the minutes of the first meeting, Conference on Bone and Wound Healing, Josiah Macy, Jr. Foundation, New York, September, 1942.
49. Reifenstein, E. C., Jr., and Albright, F.: The metabolic effects of steroid hormones in osteoporosis, *J. Clin. Investigation* **26**: 24–56, 1947.
50. Schenker, V., and Browne, J. S. L.: Comment on (48), p. 44 of ref. (48).
51. Abels, J. C.; Young, N. F.; Taylor, H. C., Jr., and Rhoads, C. P.: The effects of testosterone, testosterone propionate, and pregnenolone on protein fabrication in man, (abstract) *Endocrinology* **35**: 213, 1944.
52. Clarke, E., and Selye, H.: Overt and masked manifestations of folliculoid hormones, *J. Pharmacol. & Exper. Therap.* **78**: 187–196, 1943.
53. Albert, S., and Selye, H.: The effect of various pharmacological agents on the morphogenetic actions of estradiol, *J. Pharmacol. & Exper. Therap.* **75**: 308–315, 1942.

54. Gasche, P., and Schuler, W.: Zur Wirkung von Pregnenolone (Δ^5 Pregnen-ol-(3β)-on) und Testosteronpropionat (Perandren) auf Rattenorgane, *Helvet. phys. and pharm. acta* **4:** C 11–12, 1946.
55. Leathem, J. H., and Brent, B. J.: Influence of pregneninolone and pregnenolone on spermatogenesis in hypophysectomized adult rats, *Proc. Soc. Exper. Biol. & Med.* **52:** 341–343, 1943.
56. Albert, S., and Selye, H.: On the gonad-protecting effect of various steroid compounds, *Canad. M. A. J.* **46:** 187, 1942.
57. Simpson, M. E.: Unpublished data.
58. Paschkis, K.: *in* discussion of Mason, H. L.: Urinary steroids in adrenal disease and the metabolism of adrenal hormones, *Recent Progr. Hormone Research* **3:** 103–123, 1948.
59. Masson, G.: Spermatogenic activity of various steroids, *Am. J. M. Sc.* **209:** 324–327, 1945.
60. Masson, G.: The spermatogenic activity of Δ^5 pregnenolone and of its esters, *Am. J. M. Sc.* **212:** 1–6, 1946.
61. Heller, C. G.: Personal communication.
62. Selye, H., and Albert, S.: Morphogenetic actions of various steroids in the castrate male rat, *J. Pharmacol. & Exper. Therap.* **76:** 137–148, 1942.
63. Masson, H.; Borduas, A., and Selye, H.: The sex hormone actions of some steroids related to desoxycorticosterone and progesterone, *Rev. canad. de biol.* **1:** 57–64, 1942.
64. Clarke, E., and Selye, H.: The action of steroid compounds on the vaginal epithelium of the rat, *Am. J. Med. Sci.* **204:** 401–409, 1942.
65. Selye, H., and Clarke, E.: Ovarian function in hypophysectomized rats, *Anat. Rec.* **86:** 393–402, 1944.
66. Reynolds, S. R. M.: Cholinergic action of oestrin, *Science* **87:** 537, 1938.
67. Torda, C., and Wolff, H. G.: Effect of steroid substances on synthesis of acetylcholine, *Proc. Soc. Exper. Biol. & Med.* **57:** 327–330, 1944.
68. Masson, G., and Selye, H.: Response of metrial gland to treatment with various steroids, *Endocrinology* **31:** 549–552, 1942.
69. Selye, H., and Masson, G.: Studies concerning luteoid action of steroid hormones, *J. Pharmacol. & Exper. Therap.* **77:** 301–309, 1943.
70. Selye, H., and Albert S.: Prevention by certain steroids of testicular atrophy usually elicited by small doses of testosterone, *Proc. Soc. Exper. Biol. & Med.* **49:** 227–229, 1942.
71. Goldzieher, M. A., and Goldzieher, J. W.: Toxic effects of percutaneously absorbed estrogens, *J.A.M.A.* **140:** 1156, 1949.
72. Selye, H.: Anesthetic effect of steroid hormones, *Proc. Soc. Exper. Biol. & Med.* **46:** 116–121, 1941.
73. Kochakian, C. D.: The effect of various steroid hormones on the "alkaline" and "acid" phosphatases of the kidney of the mouse, *Am. J. Physiol.* **145:** 118–122, 1945.
74. Oster, K. A.: On the tissue aldehyde shift in the rat kidney, *Endocrinology* **37:** 69–76, 1945.
75. Dean, A. L.; Abels, J. C., and Taylor, H. C.: The effects of certain hormones on the renal function of man, *J. Urol.* **53:** 647–651, 1945.
76. White, H. L.; Heibecker, P., and Rolf, D.: Some endocrine influences on renal function and cardiac output, *Am. J. Physiol.* **149:** 404–417, 1947.
77. Bruzzone, S., and Lopez, H.: Comparative cortical action of different preg-

nenolones in the adrenalectomized guinea pig, *Proc. Soc. Exper. Biol. & Med.* **68:** 579–581, 1948.

78. SELYE, H.: On the hormonal activity of a steroid compound, *Science* **94:** 94, 1941.
79. HOAGLAND, H.: Personal communication, June, 1946.
80. ELLINGER, F.: Influence of pharmacological agents on effects of irradiation, *Radiology* **50:** 234–243, 1948.
81. HOAGLAND, H.: The human adrenal cortex in relation to stressful activity, *J. Aviation Med.* **18:** 450–464, 1947.
82. PINCUS, G., and HOAGLAND, H.: Fatigue and the adrenal cortex (Society Proceedings) *J. Nerv. & Ment. Dis.* **104:** 442–447, 1946.
83. PINCUS, G., and HOAGLAND, H.: Steroid excretion and stress of flying, *J. Aviation Med.* **14:** 173–193, 1943.
84. IGLESIAS, R., and BRUZZONE, S.: On the antitumorigenic action of large quantities of pregnenolones, *Proc. Soc. Exper. Biol. & Med.* **68:** 579–581, 1948.
85. MASSON, G.: Personal communication, May, 1946.
86. GRAHAM-BRYCE, I.; BULLEN, A. K., and FORBES, W. H.: Effect of pregnenolone on the ability to perform prolonged psychomotor tests, *Psychosom. Med.* **7:** 353–538, 1945.
87. Anonymous communication.
88. SCHWAB, R. S.: Unpublished data.
89. LANSBURY, J.: Personal communication, March, 1947.
90. DAVISON, R. A.; KOETS, P., and KUZELL, W. C.: Excretion of 17-ketosteroids in ankylosing spondylarthritis and in rheumatoid arthritis; a preliminary report, *J. Clin. Endocrinol.* **7:** 201–204, 1947.
91. DAVISON, R.; KOETS, P.; SNOW, W. G., and GABRIELSEN, L. C.: Effects of Δ^5-pregnenolone in rheumatoid arthritis, *Arch. Int. Med.* **85:** 365–388, 1950.
92. KEARNS, W. M.: Personal communication; February, 1950.
93. GUEST, C. M.; KAMMERER, W. H.; CECIL, R. L., and BERSON, S. A.: Observations on the use of epinephrine, pregnenolone and testosterone in the treatment of rheumatoid arthritis (to be published).
94. COHEN, A.: Personal communication, August, 1949; February, 1950.
95. COHEN, A.; GOLDMAN, J.; DUBBS, A. W., and MCBRIDE, T. J.: Remissions in rheumatoid arthritis following gold therapy: with preliminary report of 20 patients treated with Δ^5 pregnenolone (to be published).
96. COHEN, A.: Δ^5 Pregnenolone in rheumatoid arthritis (to be published).
97. WARTER, P. J.: Personal communication, August, 1949.
98. ISHMAEL, W. K., HELLBAUM, A.; KUHN, J. F., and DUFFY, M.: The effects of certain steroid compounds on various manifestations of rheumatoid arthritis, *J. Oklahoma State M. A.* **42:** 434–437, 1949.
99. ISHMAEL, W. K.: Personal communication, October, 1949.
100. MANNING, R. A.: Personal communication, December, 1949.
101. MANNING, R. A., and BRUGSCH, H. G.: Personal communication, January, February, 1950.

The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Drs. Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Postgraduate Course in Endocrinology

AMERICAN COLLEGE OF PHYSICIANS

May 15–20, 1950

University of Illinois College of Medicine, Chicago, Illinois

W. O. Thompson, M.D., F.A.C.P., Director

(Minimal Registration, 50; Maximal Registration, 100)

Fees: A.C.P. Members, $30.00, Non-members, $60.00

THIS course will provide an intensive review of recent developments in the field of endocrinology, including endocrine gynecology and sterility. A considerable amount of time will be devoted to the clinical uses of ACTH and Cortisone and related steroids. Special attention will be paid to clinical disorders, and physiological and biochemical developments will be presented in relation to their bearing on the interpretation of clinical phenomena.

Leading teachers from all over the United States and Canada will take part in the course. Endocrine disorders will be illustrated by the presentation of patients during the last hour of each afternoon session. Men on the program will take part in the discussion of these cases. There will be a Round-table Discussion each day at luncheon in which all men on the faculty that day will participate. This will provide an opportunity for the men taking the course to discuss various problems informally with the teachers. There will be morning and afternoon sessions each day except on Saturday, when there will be a morning session only.

Meeting Place: Illinois Room, La Salle Hotel, Chicago. The physical facilities for the course are excellent. The Management has assured us that they will make every effort to serve us well. It has been our experience that it is more convenient for the men taking a postgraduate course of this type to have it in a hotel rather than in the medical school. Everything is under one roof, and no time is lost in transportation.

Hotel Accommodations: Full arrangements have been concluded for the housing of the registrants, as well as the housing of the course itself, at the La Salle Hotel, La Salle St., at Madison St., Chicago, Mr. M. P. Mathewson, Sales Director. The hotel announces that those who plan to attend the course would have a better choice of accommodations if they would plan to arrive on Sunday, May 14. Rates: Single rooms, $5.00–$8.00; double rooms, $7.00–$10.00; twin-bedded rooms, $9.50–12.00; two rooms connecting bath for 4, $13.50–$15.00; suites, $9.50–$25.00. When writing for accommodations, address request to Mr. Mathewson and identify yourself with this course and with the College.

Intermissions: There will be a short intermission every morning and afternoon during which light refreshments will be served at a nominal charge.

Luncheons: Luncheon will be served in the Century Room on Monday, Tuesday and Friday, May 15, 16 and 19, and in the Grand Ballroom on Wednesday and Thursday, May 17 and 18. Tickets will be available at the registration desk. It is suggested that registrants buy tickets for all intermissions and all luncheons at one time.

(For program, *see* March 1950 issue of the Journal)

The Laurentian Hormone Conference

THE Laurentian Hormone Conference of the A.A.A.S. will hold its 1950 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 10 to 15. The following program has been arranged:

I. Gonad Development and Function

A. Embryogenesis of the adrenal and the reproductive glands.
Dr. Emil Witschi, The State University of Iowa
Sunday evening, September 10

B. Mammalian spermatogenesis.
Dr. Warren O. Nelson, The State University of Iowa
Monday morning, September 11

C. Oogenesis in the mature ovary.
Dr. S. Zuckerman, University of Birmingham, England
Monday morning, September 11

II. The Use of Isotopes in Hormone Problems

D. Studies in the metabolism of steroid hormones.
Dr. T. F. Gallagher, Sloan-Kettering Institute
Monday evening, September 11

E. Biosynthesis of steroids.
Dr. Konrad Bloch, The University of Chicago
Tuesday morning, September 12

III. Hormones and Metabolism

F. Parameters of metabolic problems.
Dr. Dwight J. Ingle, The Upjohn Company
Tuesday morning, September 12

G. Metabolic processes influenced by the growth hormone.
Dr. F. J. Young, Biochemical Laboratory, Cambridge, England
Tuesday evening, September 12

H. The problem of adrenal androgens in man.
Dr. Allan T. Kenyon, The University of Chicago
Wednesday morning, September 13

IV. Chemistry, Physiology and Clinical Endocrinology of the Adrenal Cortex

I. Chemistry of adrenal cortex steroids.
Dr. P. L. Julian, The Glidden Company
Wednesday morning, September 13

J. The nature of adrenal secretory activity.
Dr. Oscar Hechter, The Worcester Foundation for Experimental Biology
Thursday morning, September 14

K. The adrenal cortex in salt and water metabolism.
Dr. Robert Gaunt, Syracuse University
Thursday morning, September 14

L. A consideration of the role of the adrenal cortex in the regulation of certain metabolic processes.
Dr. Frank L. Engel, Duke University
Thursday evening, September 14

M. Observations on the physiological effects of cortisone and ACTH in man.
Drs. R. G. Sprague, M. H. Power and H. L. Mason, The Mayo Clinic
Friday morning, September 15

V. Hormone Therapy in Cancer

N. Experimental studies with ACTH and cortisone in patients with neoplasms.
Dr. O. H. Pearson, Sloan-Kettering Institute
Friday morning, September 15

O. Steroid hormone therapy in cancer.
Dr. I. T. Nathanson, Massachusetts General Hospital
Friday evening, September 15

Attendance at this Conference is limited by the accommodation available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, E. R. Squibb & Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst & Co.; E. C. Reifenstein, Jr., Sloan-Kettering Institute; A. White, School of Medicine, University of California at Los Angeles; and G. Pincus, Chairman, The Worcester Foundation for Experimental Biology. Application blanks for attendance at the Conference may be obtained from the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. *Applications to be considered by the Committee must be received by May 20, 1950.*